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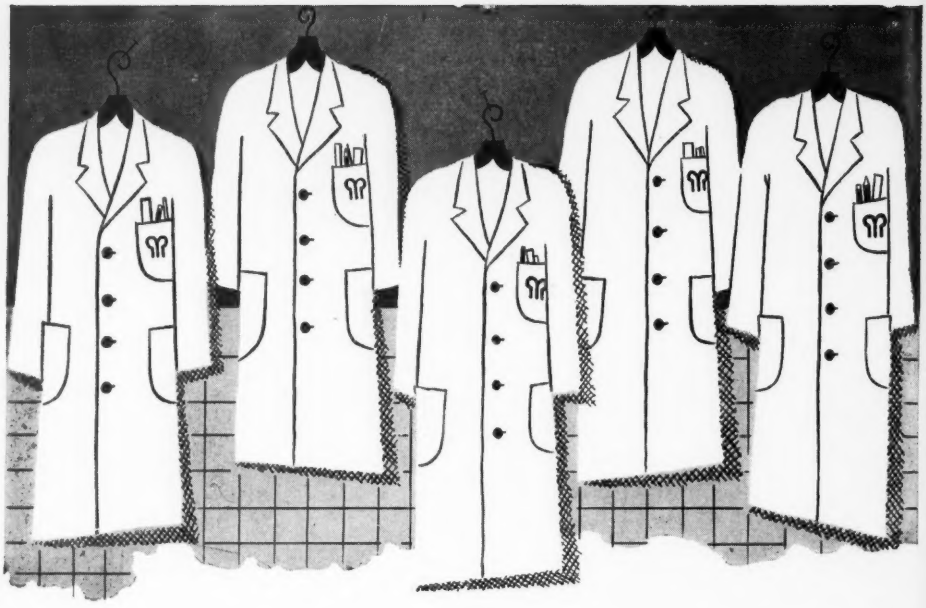

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TESTICULAR DEFICIENCY: A CLINICAL AND PATHOLOGIC STUDY*†

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SINCE the introduction into clinical medicine of the testicular biopsy by Hotchkiss (1) and by Charny (2) much progress has been made in elucidating the pathogenesis of various types of testicular deficiency. Some clinicians have been chiefly concerned with the problem of sterility (1, 2, 3, 4, 5, 6). Others have studied syndromes with endocrinologic manifestations, such as eunuchoidism (7, 8, 9), a testicular syndrome associated with gynecomastia (10, 11, 12), and the male "climacteric" (13, 14, 15). Nelson (16, 17) has made a general review of the subject of hypogonadism and more recently other general papers (18, 19, 20, 21, 22) have become available.

The present study is an attempt to elucidate the various syndromes associated with testicular deficiency, from three angles: clinical status, tes-

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ticular histology as determined by biopsy, and endocrinologic pattern as judged by hormonal assay.

CLINICAL MATERIAL

In the eight years up to 1947, at the Massachusetts General Hospital, each of 256 persons on one or more occasions was subjected to testicular biopsy. A preliminary report has already been made on some of these

TABLE 1. ANALYSIS OF AVAILABLE MATERIAL—221 CASES

Cases excluded for following reasons:		
Incomplete data	17	
Biopsy specimen inadequate for diagnosis	14	
Testicular infections	6	80
General debilitation	5	
Previous endocrine therapy	3	
Obstructed excretory passages (*e.g.*, vasa)	35	
Cases analyzed in detail (*see* Table 2)		141
		221

cases (23). In addition, testicular tissue from patients at autopsy and after castration has been studied.

Material from certain types of patients, notably those exhibiting testicular neoplasms and sexual precocity, has been excluded as not being pertinent to the present study. There remained 221 cases. An additional 80 cases have been excluded for various reasons (Table 1). Of these, attention is called to an interesting subgroup characterized by bilateral obstructions (sometimes congenital anomalies) of the vasa or of the epididymes and by azoospermia with an essentially normal testicular histologic picture. This condition was present in approximately one third of the patients complaining of sterility. It has been frequently cited to illustrate the diagnostic value of the testicular biopsy (1, 5, 6, 23, 24, 25). This clinically important but academically straightforward subgroup will not be further discussed in the present paper.

Thus there remained 141 cases of testicular deficiency for analysis.

LABORATORY METHODS

1. Examination of seminal fluid

Semen was obtained by masturbation into a glass jar, and was studied in the usual manner (26, 27). This examination was repeatedly made on

men complaining of sterility, but it was rarely possible in patients with eunuchoidism, as they seldom have ejaculations.

2. Urinary 17-ketosteroid assays

The levels of urinary 17-ketosteroids were determined by a modification of the Zimmermann reaction (28). Averages of determinations made during a clinical state comparable to that at the time of testicular biopsy are reported in this paper. The range for normal men lies between 8 mg. and 18 mg. per day.

3. Urinary follicle-stimulating hormone (FSH) assays[1]

The amount of FSH in the urine by the method used in this clinic (29) was estimated on graded dilutions of extracts of alcoholic precipitates. The biologic assay was carried out on intact infantile mice, a significant enlargement of the uterus being the criterion of a positive result. In the accompanying tables are shown only the highest levels of positive responses and the lowest levels of negative (*i.e.*, absent) responses. By the "dialysis method," which is primarily designed to differentiate between normal and subnormal levels of FSH, the range for positive responses is from 6.5 to 26 mouse units (m.u.) per day; *i.e.*, negative values at 6.5 m.u. are abnormally low, and positive values at 52 m.u. or above are abnormally high. By the "non-dialysis method," which is designed primarily to differentiate between normal and high levels of FSH, positive responses at 10 m.u. per 100 cc. or at 96 m.u. per day are abnormally high.

4. Testicular biopsy

This was done in the majority of cases by one of us (F. A. S.) after the technic of Charny (2). Either general anesthesia was used, or, in certain cases with sufficiently large testes, subcutaneous novocaine injections. Often a biopsy specimen was taken from each testis. However, in many cases of eunuchoidism with unusually small testes the operation was limited to one gonad. With the use of curved iris scissors a slice measuring approximately 4 mm. $\times$ 2 mm. $\times$ 2 mm. was excised from the substance of the gland. Hemostasis was controlled by a single cutaneous suture.

The biopsy specimens were immediately placed in a fixative. Zenker's solution with 5 per cent glacial acetic acid or Helly's fluid seemed to be the most satisfactory fixatives for routine use. Formalin fixation was employed only in the technics requiring frozen sections, as the after-treat-

[1] As will be discussed later, it is not certain whether the test for FSH determines FSH alone or FSH and LH. However, the authors believe that the reader will not be badly misled if he considers the FSH test as representing FSH alone.

ment with alcohol in paraffin sections produced serious shrinkage of the Sertoli cell cytoplasm. The sections were stained routinely with phloxine-methylene blue or hematoxylin-eosin, phosphotungstic acid hematoxylin, and Mallory's aniline blue or Masson's trichrome connective tissue stains. Other methods were employed to elucidate certain points—for example, the periodic acid-leuko-basic fuchsin technic as developed by McManus (30) for basement membrane.

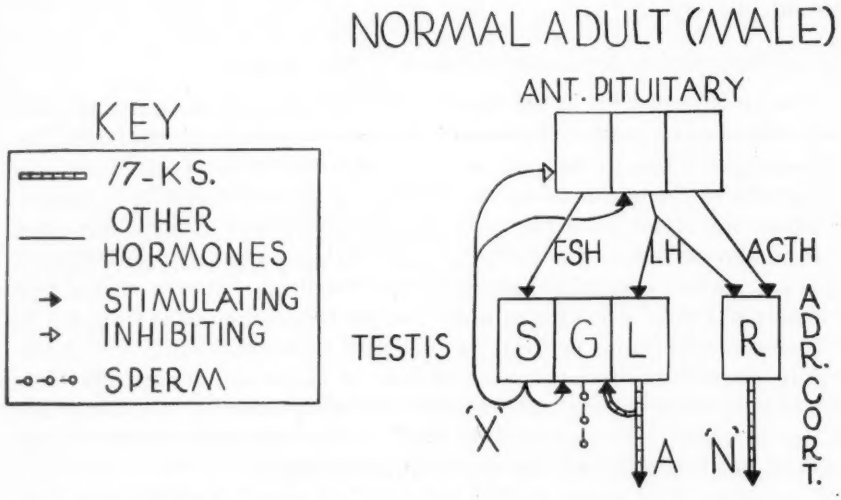

FIG. 1. *Schematic diagram to indicate authors' present conceptions of normal tissue-hormonal interrelations.*

For discussion, *see* text. FSH = follicle-stimulating hormone; LH = luteinizing hormone; ACTH = adrenocorticotropic hormone; S = Sertoli cells; G = germinal epithelium; L = Leydig cells; R = reticular zone; 'X' = 'X' hormone; A = androgenic hormone; 'N' = nitrogen hormone.

It is probable that the Leydig cell hormone inhibits LH secretion and this should be indicated by an inhibiting arrow; for simplification this arrow is omitted.

Present beliefs of authors as to pertinent tissue-hormonal interrelations

Figure 1 is introduced here to serve as a brief orientation of the pertinent tissue-hormonal interrelations. These concepts are not to be considered as final; some will be supported by the data to follow and will be reconsidered later.

The testis (Fig. 1) is divided into three compartments: S—the cells of Sertoli, G—the germinal epithelium, and L—the cells of Leydig. The

Sertoli cells have two functions: to afford mechanical support for the germinal epithelium and to manufacture a second testicular hormone, the "X" hormone. The cells of Leydig produce the male androgenic hormone, A, presumably testosterone or some closely related steroid.

The adrenal cortex has one component which concerns the present discussion. The cells of the reticular zone (see Fig. 1-R) are probably closely akin to the cells of Leydig. Both have a common embryologic origin; both have a similar life history (present during fetal life, involuting in large measure during the first few weeks of life, and redeveloping at puberty (31)); both produce steroids which are precursors of urinary 17-ketosteroids (17-ks.).

The anterior pituitary produces 4 tropic hormones which may bear on the subject. No attempt is made in Figure 1 to indicate from which anterior pituitary cells the individual hormones originate.

The follicle-stimulating hormone (FSH) is intimately connected with the testicular tubule, where it probably stimulates the Sertoli cells (less likely the germinal cells) to produce "X" hormone. The "X" hormone is the analogue of estrogen in the female and, like estrogen, inhibits FSH and stimulates the luteinizing hormone. Furthermore, it may play a part in the maintenance of spermatogenesis.

The luteinizing hormone (LH, interstitial-cell-stimulating hormone, ICSH) stimulates the cells of Leydig to produce testosterone. It also has an effect on the testicular tubules somewhat similar to that of FSH. Since testosterone alone also stimulates the tubules, this effect of LH may be due to production of testosterone (32).

The lactogenic hormone (luteotropin) has no known function in the male but may be concerned with LH in the stimulation of the cells of Leydig. It is omitted from Figure 1.

The adenocorticotropic hormone (ACTH) plays an important role in the stimulation of the reticular cells of the adrenal cortex to produce the adrenal cortical anabolic ("nitrogen" or "N") hormone (33, 34, 35, 36), which gives rise to part of the 17-ketosteroids excreted in the urine. Whether other tropic hormones of the pituitary, notably LH, are necessary for the production of "N" hormone remains undecided (37, 38).

RESULTS

From the levels of the urinary FSH assays as the primary consideration, the 141 cases have been classified into 3 major groups: those with low, normal, and high urinary FSH excretions, respectively. Group B with normal FSH excretion was further subdivided into those with endocrine disturbance and those without endocrine disturbance. In certain instances, in which the other clinical or histologic data strongly pointed to a case be-

longing to a different group than indicated by the FSH assay, exceptions were made. The 3 major groups are comprised of 15 conditions (Table 2).

Group A. Conditions with Low FSH Excretion

The 37 patients in this group have been subdivided according to etiology

TABLE 2. CLASSIFICATION OF 142 CASES* OF TESTICULAR DEFICIENCY

Group A. Conditions with low FSH excretion		
i. Idiopathic eunuchoidism-with-low-FSH	19	
ii. Secondary to organic lesions in or near the pituitary	13	
iii. Secondary to estrogen therapy	5	
		37
Group B. Conditions with normal FSH excretion		
(a) With endocrine disturbance		
iv. Lack of LH secretion?	5	
v. Acromegaly	3	
(b) Without endocrine disturbance		
vi. Arrest of maturation in spermatogenesis	8	
vii. Hypospermatogenesis	15	
viii. Oligospermia with normal testicular histology	9	
		40
Group C. Conditions with high FSH excretion		
ix. Absence of germ cells	19	
x. Sclerosing tubular degeneration	30	
xi. Absence of testes	4	
xii. Normal testicular histology (? climacteric)	6	
xiii., xiv., xv. Miscellaneous conditions	6	
		65
		142

* There were actually only 141 patients, but 1 patient with acromegaly was listed twice as Case 33 and 43 because of a change in his status following estrogen therapy.

(Table 2). Included were: i. 19 cases in which the low FSH was idiopathic, ii. 13 cases in which it was secondary to an organic lesion in or near the pituitary, and iii. 5 cases in which the low FSH followed estrogen therapy.

i. *Idiopathic eunuchoidism-with-low-FSH*

The important data on the 19 cases of this condition, which is the common but not the only form of eunuchoidism, are presented in Table 3.

Cases 1–5 represented examples of this condition in its purest form. The typical clinical findings were: ejaculations absent; voice high, and thyroid

cartilage small; extremities long with span exceeding height by several inches; no temporal recession of the hairline; no beard; hair scanty on the trunk and extremities; axillary hair sometimes present, sometimes absent; pubic hair present, though usually scant as in a young adolescent boy or

TABLE 3. GROUP A. CONDITIONS WITH LOW FSH EXCRETION

i. *Idiopathic Eunuchoidism-with-Low-FSH*

	Case No.	Age (yrs.)	Pubic hair*	17-ks. (urinary) mg./day	FSH (urinary)** m.u./day	Path. No.	Sertoli cell maturation†	Germ cell proliferation	Tunica propria thickening	Peritubular proliferation	Leydig cells‡	Degenerative changes
(a)												
Primary lack of FSH	1.	16	+	2.9	*−6*	45-5096	0	0	0	0	0	None
	2.	18	+	2.0	*−6*	47-5410	0	0	0	0	0	None
	3.	37	+	4.4	*−6*	43-5396	0	0	0	0	0	None
	4.	20	+	9.8	*−6*	47-4096	0	0	0	0	0	None
	5.	19	+	9.5	*+3–6*	45-5062	+	+	0	0	0	None
(b)												
Primary lack of FSH & LH	6.	19	0	0.7	*−6*	41-109	0	0	0	0	0	None
	7.	21	0	?	*−6*	41-7751	0	0	0	0	0	None
	8.	40	0	0.4	*−3*	41-1421	+	0	0	0	0	None
(c)	9.	20	+	4.0	*−6*	46-1784	+	+	+	0	0	Slight
Diminution of FSH & ? of LH after puberty	10.	24	+	5.9	*−3*	44-1881	+	+	+	0	0	Slight
	11.	20	+	2.6	*−3*	46-9598	+	0	++	0	0	Slight
	12.	19	+	5.9	*+6–13*	46-6772	+	+	++	0	+	Slight
	13.	18	+	4.5	*+13–26*	43-3129	+	+	+	0	0	Slight
	14.	21	+	3.5	*+6–13*	43-4645	+++	++	+	+	Abn.	Slight
	15.	47	+	9.8	*−6 & +6*	43-5880	+++	++	+	+	Abn.	Slight
	16.	28	+	5.2	*+3–6*	47-6143	+++	++	0	+	Abn.	Slight
	17.	48	+	4.5	*−3*	44-1142	+++	++	+	+	0	Slight
	18.	35	+	4.7	*−6*	46-3440	+++	+	+	+	0	Slight
	19.	34	+	10.8	*−6*	45-1280	+++	+	++	++	0	Moderate

* The presence of pubic hair is indicated by a plus sign.

** Results of urinary FSH assays are condensed so that the highest level showing a positive response is preceded by a plus sign and the lowest level giving a negative response is preceded by a minus sign. If results were negative at all levels the figure shown is the lowest at which bioassay was performed. For the sake of brevity, tests at 6.5 m.u. are recorded as 6 m.u. The use of *italics* indicates that the assay was done by the dialysis method, and the use of plain type, the non-dialysis method. In the following tables a few FSH results, specially indicated, are reported in m.u. per 100 cc.

† The histologic details are described in quantitative gradations from 0 to +++. Thus, a normal adult should have +++ for Sertoli cell maturation, germ cell proliferation and Leydig cells, and should have 0 for tunica propria thickening and peritubular proliferation.

‡ Abn. means abnormal.

girl; testes, phallus and prostate infantile in size; moderate obesity, frequently but not invariably; no gynecomastia; normal sella turcica by x-ray examination. There was clinical and laboratory evidence to rule out panhypopituitarism. The 17-ketosteroid levels ranged between 2.0 mg. and 9.8 mg. per day, that is, from the level found at about 11 years of age to that of an adult (39). The urinary FSH assays were invariably negative at 6.5 m.u. per day.

The testicular structure in Cases 1–4 was strikingly similar to that of a prepubertal child (*cf.* Fig. 4 with Fig. 2). Thus the tubules were represented by lumenless cords in which there was no differentiation of the Sertoli cells, and the germ cells showed little or no mitotic activity. No Leydig cells were present. Thickening of the tubular tunica propria and deposition of peritubular collagen and elastic tissue, which we interpret as degenerative changes (*see* subsequent discussion), were absent. The testis in Case 5 was essentially similar except that there was slight maturation of Sertoli cells and slight increase in mitotic activity of the germ cells.

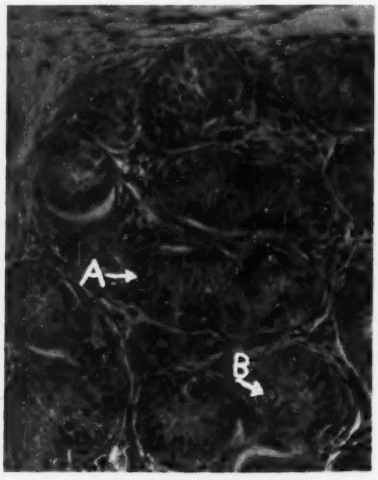

FIG. 2. Subcapsular zone of normal testis at six months. Note small lumenless tubules made up of a syncytium containing undifferentiated nuclei and a few inactive primitive germ cells. No recognizable Leydig cells in the interstitial tissue. A = undifferentiated nuclei; B = germ cell. ×150.

The disorder in Cases 1–5 is interpreted as due to a lack of FSH secretion and in some instances to a diminution of LH as well. Adrenal androgenic secretion occurred at about the normal age as judged by the time of appearance of pubic hair. The 17-ketosteroid excretion, however, was low (less than 4.5 mg.) in 3 of the 5 cases, which suggests that the reticular zone of the adrenal cortex is also deficient in some if not all of these cases. Whether this second deficiency is the result of a lack of ACTH or of some other tropic hormone such as LH is uncertain. The following sequence is suggested: primary underproduction of FSH, decreased "X" hormone, decreased LH, and decreased stimulation of Leydig cells and the reticular zone of the adrenal cortex (*see* diagram C in Fig. 17). Another possibility

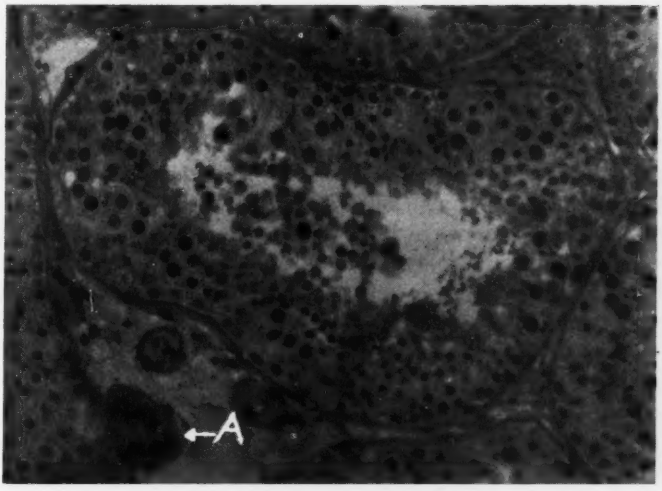

FIG. 3. Normal adult testis with active spermatogenesis and small islands of Leydig cells. A = Leydig cells. ×250.

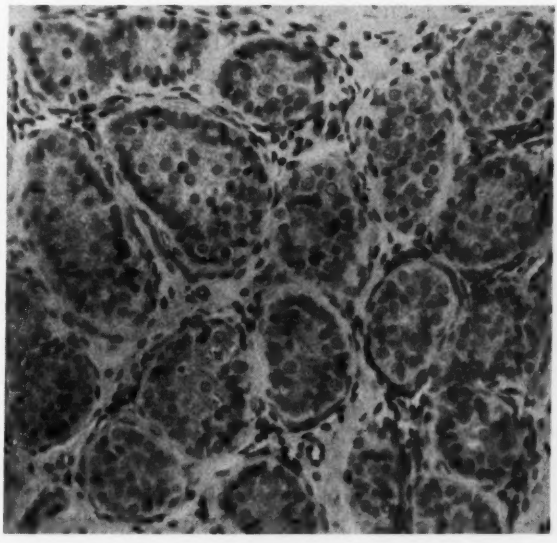

FIG. 4. Case 7. Histologic section of testicular biopsy specimen in a man aged 21 years with eunuchoidism-with-low-FSH. Immature tubules and no Leydig cells. Compare with Figure 2. ×250.

is that LH may be low for the same unknown reason that FSH is low. Just why the Leydig cells are absent rather than just diminished, if either of the above explanations is correct, is not apparent but possibly the absence of FSH secretion is the reason (*see* subsequent discussion).

Cases 6–8 were separated from Cases 1–5 because the patients exhibited complete absence of pubic and axillary hair and of urinary 17-ketosteroids. In other respects the findings were similar, *i.e.*, FSH assays of less than 6.5 m.u. per day, and biopsy specimens showing no evidence of pubertal changes in the testes (Fig. 4).

In this group, then, adrenal androgenic function is apparently absent rather than diminished. This is interpreted as indicating a complete lack of LH as well as of FSH, and the condition is comparable to that in a prepubertal boy (diagram B in Fig. 17).

Cases 9–19 were separated from Cases 1–8 because the testes showed by biopsy, evidences of tubular stimulation, past or present. A notable feature was the thickening of the tubular tunica propria exhibited by 10 of the 11 cases. This was likewise noted (*see* following sections) in many of the cases with acquired destructive lesions of the hypophysis and is regarded as a degenerative change.

All except 2 of the 11 patients had low 17-ketosteroid assays. Although 3 had normal or low normal FSH excretions, in view of the other findings, they were left in this group.

The first 5 of this group differed slightly from the last 6, in that they were younger, had less Sertoli cell maturation and showed no peritubular proliferation. The last 6 showed tubules of adult dimensions with mature Sertoli cells and slightly or moderately active spermatogenesis. There was also in these 6 cases evidence of peritubular proliferation of the elastic and fibrous tissue, which is regarded as further evidence of degeneration. Furthermore, although in all of the first 5 patients Leydig cells were absent, 3 of the last 6 showed some Leydig cells, albeit degenerate ones. These cells were large and polygonal with foamy cytoplasm which often contained an unusual number of Reinke crystalloids. As clinical evidence of greater gonadal function in the past was the fact that 4 of the last 6 subjects, though impotent at the time of study, had been married and were allegedly potent.

The endocrine disorder in Cases 9–19 is considered to be qualitatively similar to that in Cases 1–5 (diagram C in Fig. 17). The quantitative variations within this group itself and between it and Cases 1–5 are thought to portray differences in the amount and duration of FSH secretion. In all cases this secretion, though perhaps present to a slight degree at the time of study, is considered as being less than it had been at some previous

time. The presence of Leydig cells, even though abnormal ones, in 3 of the subjects suggests the presence of some LH secretion.

ii. *Low FSH secondary to organic lesions in or near the pituitary*

This condition differs from the preceding one chiefly as regards the cause of the failure of FSH secretion; here the initial lesion was demonstrable—a tumor, cyst, or traumatic lesion of the pituitary or of the juxtasellar region. Furthermore, the low 17-ketosteroid excretion in this group was almost certainly due to direct involvement of the tropic hormone in ques-

TABLE 4. GROUP A. CONDITIONS WITH LOW FSH EXCRETION

ii. *Secondary to Organic Lesions in or Near the Pituitary*

	Case No.	Age (yrs.)	Pubic hair	17-ks. (urinary) mg./day	FSH (urinary) m.u./day	Path. No.	Sertoli cell maturation	Sertoli cell lipoid content*	Germ cell proliferation	Tunica propria thickening	Peritubular proliferation	Leydig cells	Degeneration changes
(a)	20.	19	+	2.1	*−3*	47-3447	0	0	0	+	0	0	Slight
FSH & ?LH decreased	21.	28	+	2.4	*−6*	47-5128	++	0	0	++	0	0	Moderate
during puberty	22.	23	+	8.7	*−3*	47-3726	++	0	0	+	+	+	Moderate
	23.	26	+	4.0	*−6*	43-8012	++	0	+	++	+	0	Moderate
(b)	24.	74	+	2.2	*−3*	A-11926	+++	+	0	+++	+++	0	Marked
Diminution of FSH &	25.	72	+	1.3	−10/100 cc.	A-9945	+++	++	0	+++	+++	0	Marked
? of LH during adult	26.	64	+	0.6	*−3*	44-1102	+++	+++	+	++	+++	Abn.	Moderate
life	27.	27	+	1.8	Not done	A-10092	+++	++	+	++	+++	Abn.	Moderate
	28.	56	+	1.8	Not done	A-9238	+++	+++	0	++	+++	Abn.	Moderate
	29.	52	+	2.2	*−3*	42-7082	+++	+++	+	++	+++	Abn.	Moderate
	30.	49	+	4.2	*−3*	44-98	+++	+++	+	++	+++	Abn.	Moderate
	31.	53	+	1.0	*+6 & −6*	42-1914	+++	+	++	+	+	Abn.	Slight
	32.	41	+	1.1	*+3-6*	47-147	+++	+	++	+	+	+	Slight

* The normal for "Sertoli cell lipoid content" would be 0, and maximal lipoid is represented by +++.
For key to other signs and abbreviations, *see* footnote to Table 3.

tion (?LH) rather than to a failure of "X" hormone to stimulate LH. Indeed many of these cases were suffering from destruction of all pituitary functions, *i.e.*, they had panhypopituitarism. In the case of a juxtasellar cyst it is possible that the resulting endocrine disturbance is due to interference with the hypothalamic-pituitary nervous-humoral pathway rather than to destruction of the hypophysis.

The data on Cases 20–32 are summarized in Table 4. In 9 of the 13 subjects the pituitary lesion was verified at operation or autopsy; in the others there was radiologic evidence of a sellar abnormality. In many, on clinical examination there were local signs of expanding intrasellar or suprasellar processes.

The testicular lesions appeared to depend on the stage of testicular development attained prior to the onset of gonadotropin suppression.

The findings in Cases 20–23 (scanty pubic hair, low urinary 17-ketosteroids, low FSH excretions, relatively immature seminiferous tubules with tunica propria thickening and absent Leydig cells) were strikingly similar to those in Cases 9–13 (Fig. 5). It is assumed that FSH and LH secretions had been interrupted before the completion of testicular maturity, and that the thickened tunica propria represents a degenerative change

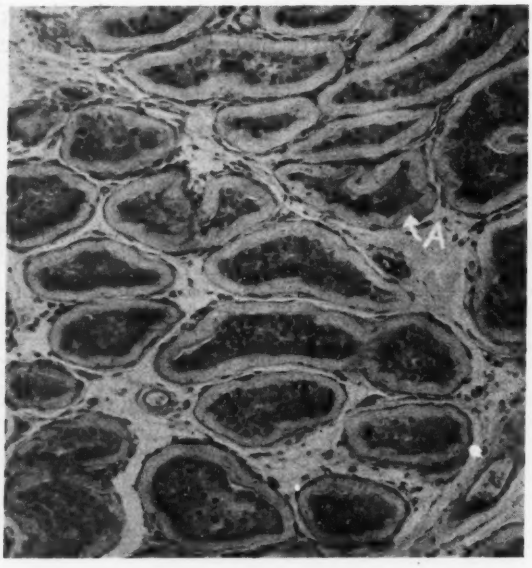

FIG. 5. Case 21. Histologic section of testicular biopsy specimen in a man of 28 years with a Rathke's pouch cyst acquired during puberty. The tubules are relatively immature and show pronounced thickening of the tunica propria. No Leydig cells are present. A = thickened tunica propria. ×130.

on the part of the tubules as a result of a failing pituitary function. This point of view, it should be remembered, led to a similar interpretation of the degenerative testicular changes in Cases 9–13.

In Cases 24–32 the patients had an average age of over 50 years. Most of these men had married and several had had children before the symptoms of pituitary lesions occurred. Therefore, their somatic and gonadal development had reached the normal adult state, but at the time of study the urinary 17-ketosteroids and FSH levels were low. Thus this group was comparable in most respects to Cases 14–19 in the group with idiopathic eunuchoidism.

The extent of testicular degeneration was thought to be roughly pro-

portional to the completeness of the pituitary destruction. Often the abnormalities were more marked than in Cases 14–19. The changes consisted of impairment of germ cell activity, tunica propria thickening, peritubular proliferation and degeneration of Leydig cells. In addition, there was a finding not seen in Cases 14–19. The cytoplasm of the Sertoli cells was filled with sudanophilic material which was not doubly refractile. These

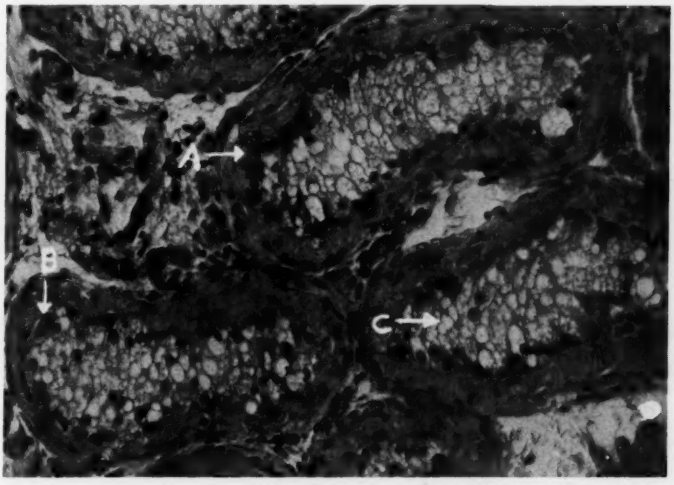

FIG. 6. Case 26. Histologic section of testicular biopsy specimen in a man of 64 years with a pituitary tumor. Note thickening of the tunica propria and multinucleated spermatogonia. Most of the nuclei are of Sertoli cell type. The Sertoli cytoplasm is occupied by droplets of sudanophilic material. A few atrophic Leydig cells were present but are not illustrated. A = thickened tunica propria; B = multinucleated spermatogonia; C = lipoid accumulation in the Sertoli cytoplasm. × 200.

swollen lipoid-filled cells often occluded the lumina of the shrunken tubules (Fig. 6). The reason for this lipoid accumulation is not understood; it may represent unused nutriment for the spermatogenic cells or material which the unstimulated Sertoli cells are unable to form into a hormone (*see* subsequent discussion).

The endocrinologic interpretation for Cases 24–32 is similar to that for Cases 20–23 except that FSH and LH were not suppressed until after both had been present in normal quantities.

iii. *Low FSH secondary to estrogen therapy*

Table 5 contains data on 5 patients in whom the testicular lesions were presumably the result of estrogen therapy. In each of them sexual develop-

ment had at one time been normal. In Cases 33 and 35 the men received estrogen therapy for acromegaly, and in Cases 34, 36 and 37, for cancer of the prostate with metastases. Prior to therapy, in Cases 33, 34 and 35, control observations had been obtained; these showed normal levels of 17-ketosteroid and FSH excretions for their respective ages. Control biopsy specimens prior to therapy were obtained in Cases 33 and 34 only; these were essentially normal. Of these 3 subjects with control observations the patient in Case 33 received 220 mg. of estradiol dipropionate during forty-two days; in Case 34, 180 mg. of estradiol benzoate during sixty-

TABLE 5. GROUP A. CONDITIONS WITH LOW FSH EXCRETION

iii. *Secondary to Estrogen Therapy*

Case No.	Clinical notes	Age (yrs.)	17-ks. (urinary) mg./day	FSH (urinary)* m.u./day	Path. No.	Sertoli cell lipoid content	Germ cell proliferation	Tunica propria thickening	Peritubular proliferation	Leydig cells	Degenerative changes
33a.	Untreated	19	10.1	+*26–52*	42-2171	0	++	0	0	+	None
b.	Estradiol—42 days	19	9.5	-*3*	43-3445	+	+	+	0	0	Slight
34a.	Untreated	78	4.2	+10/100 cc.	42-1171	0	+++	0	0	+++	None
b.	Estradiol—67 days	78	4.0	-*6*	42-3260	+++	+	++	+++	+	Moderate
c.	Estrogens—5 yrs. Untreated—1 yr.	84	5.0	+*13–96*	47-9285	0	0	+++	+++	0	Marked
d.	Untreated—2 yrs.	84		+96–192	Not done						
35a.	X-ray therapy only	45	9.8	+*6*	Not done						
b.	Stilbestrol—5 yrs.	50	5.1	-*6*	47-6958	++	0	+++	+++	0	Marked
36.	No control studies				Not done						
	Estrogens—31 mos.	78	0.7	Not done	47-4722	++	0	+++	+++	0	Marked
37.	No control studies Stilbestrol—6 mos. Untreated—6 wks.	76	2.0	Not done	Not done 47-5015	+++	+	++	+++	++	Moderate

* For key, *see* footnotes to Tables 3 and 4.

seven days; and in Case 35, diethylstilbestrol in massive doses over several years. In all 3 subjects the FSH excretions during therapy fell to less than 6.5 m.u. per day, while the 17-ketosteroid excretions in at least the first 2, remained essentially unchanged.

The testicular biopsies in all 5 cases after estrogen therapy showed degenerative changes including lipoid accumulation in the Sertoli cells similar to that seen in Cases 24–32 (*cf.* Fig. 7 with Fig. 6). Leydig cells were absent in 4 of the 5 cases.

Of special interest is Case 34, in which the biopsy showed the Leydig cells to be normal (+++) before treatment, rare (+) after sixty-seven days of treatment, and absent (0) after five years of treatment followed by

one year without treatment. In spite of the marked changes in the Leydig cells, the urinary 17-ketosteroids remained essentially constant. Thus it would appear that estrogen therapy, if sufficiently prolonged, may not only produce irreparable changes in the tubules but also in the Leydig cells. Further corroboration of the complete destruction of Leydig cells in this patient is the fact that the metastatic cancer of his prostate had not become reactivated at the end of two years after cessation of treatment,

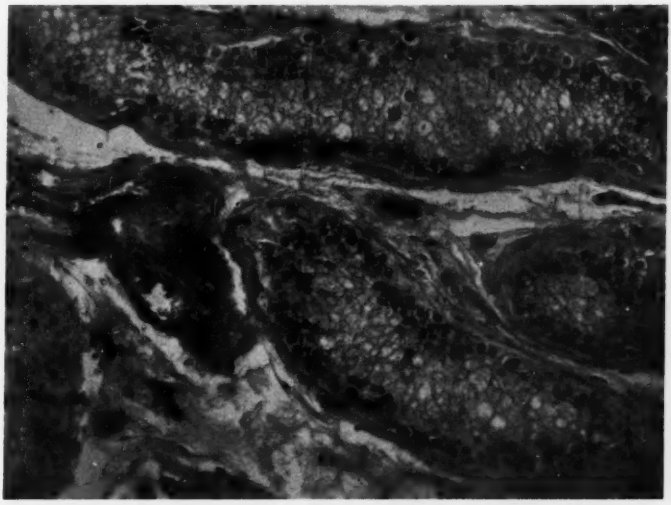

FIG. 7. Case 34. Histologic section of testicular biopsy specimen in a man of 78 years after the administration of 180 mg. of estradiol benzoate during sixty-seven days. Tubular and Leydig cell changes are similar to those in Case 26 (Fig. 6). A control biopsy specimen taken immediately before treatment showed a normal testis with active spermatogenesis and normal Leydig cells. ×155.

as judged by clinical condition and the alkaline and acid phosphatase determinations.

We, as have others before us (40, 41), ascribe the deleterious action of estrogen to its inhibition of FSH secretion with resulting tubular degeneration. The modus operandi of the disappearance of Leydig cells is most uncertain: is it inhibition of LH by estrin, absence of "X" hormone to stimulate LH, or absence of FSH to stimulate Leydig cells (*see* subsequent discussion)?

Group B. Conditions with Normal FSH Excretion

This group of 40 patients (Cases 38–77) was divided into those with endocrine disturbance and those without endocrine disturbance (Table 2).

(a) With endocrine disturbance

The data on all cases included in this subgroup are presented in Table 6

iv. *Lack of LH secretion?*

In Cases 38–41 the testicular disorder, it is believed, was due to the lack of LH secretion in the presence of normal FSH secretion. A low FSH excretion was demonstrated in only one (Case 38), that of the other 3 being normal. All 4 had radiologic evidence of pituitary or juxtasellar lesions.

TABLE 6. GROUP B. CONDITIONS WITH NORMAL FSH EXCRETION

a. WITH ENDOCRINE DISTURBANCE

iv. *Lack of LH Secretion?*

Case No.	Age (yrs.)	Pubic hair	17-ks. (urinary) mg./day	FSH (urinary)* m.u./day	Path. No.	Sertoli cell maturation	Germ cell proliferation	Tunica propria thickening	Peritubular proliferation	Leydig cells	Notes
38.	18	0	0.8?	*+3–6*	43-4596	+++	++	0	0	0	No Leydig cells
39.	46	+	3.2	*+6–13*	43-2565	+++	++	0	0	0	No Leydig cells
40.	35	+	3.3	*+6–26*	45-6070	+++	++	0	0	0	No Leydig cells
41.	55	+	3.5	*+26–52*	41-7390	+++	++	+	0	0	No Leydig cells
42.	16	0	2.4	*+13–104* –96	43-7151	++	0	0	0	0	No Leydig cells No germ cells
					v. *Acromegaly*						
43.	19	+	10.1	*+26–52*	43-2171	+++	++	0	0	+	Few Leydig cells
44.	38	+	12.2	*+26*	45-6509	+++	++	0	0	++	
45.	29	+	7.7	*+6–52*	47-1111	+++	+++	0	0	++	

* For key, *see* footnote to Table 3.

Testicular biopsies showed a nearly normal appearance of the germinal cells and Sertoli cells associated with a complete absence of Leydig cells (Fig. 8). Segaloff (42) reported a somewhat similar case with essentially the same interpretation.

The diagnosis of an FSH-producing pituitary tumor which had destroyed the remainder of the anterior pituitary, was entertained for Cases 40 and 41 in order to explain the normal FSH excretion and spermatogenesis in combination with evidence otherwise compatible with panhypopituitarism.

Case 42 was unique. In contrast to Cases 38–41 there was no radiologic evidence of a pituitary lesion. The patient was a short undeveloped 16-year-old boy who had no pubic hair, low urinary 17-ketosteroid excretion and repeatedly normal urinary FSH excretions. The testes showed maturing Sertoli cells but no germ cells and no Leydig cells. Here also the endocrine features can be interpreted as a lack of LH secretion; the cause of

the absence of germ cells remains problematic but may be an unrelated defect similar to that in certain cases described in condition ix.

v. *Acromegaly*

Cases 43–45 had definite clinical and radiologic evidence of acromegaly. Since gonadal development, and the results of urinary assays and testicular biopsies were essentially normal, they strictly do not belong in this paper.

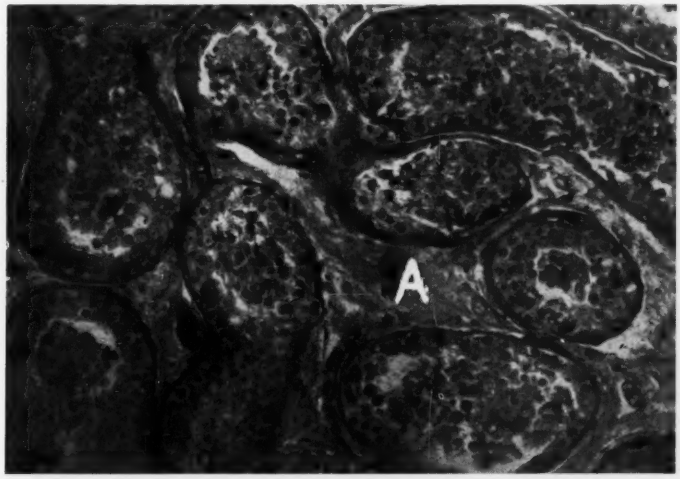

FIG. 8. Case 39. Histologic section of testicular biopsy specimen in a man of 46 years with a bullet in the pituitary region since the age of 15 years. Tubules show mild reduction in spermatogenic activity. No Leydig cells are present. A = interstitial tissue without Leydig cells. ×130.

However, they illustrate the point that pituitary lesions may exist without alteration in the secretion of gonadotropins.

(b) Without endocrine disturbance

This subgroup was comprised of 32 patients (Cases 46–77) with varying manifestations of failure of spermatogenesis, in whom there were no outward signs of endocrine dysfunction, and whose urinary assays usually gave normal results. In most instances the only complaint was infertility. This subgroup was divided into 3 fairly well-defined conditions (Table 2).

vi. *Arrest of maturation in spermatogenesis*

Cases 46–53 showed arrest at some stage of spermatogenesis (3, 6, 17). Data are summarized in Table 7. These 8 normally developed but sterile

TABLE 7. GROUP B. CONDITIONS WITH NORMAL FSH EXCRETION

b. WITHOUT ENDOCRINE DISTURBANCE

vi. *Arrest of Maturation in Spermatogenesis*

Case No.	Age (yrs.)	Size of testes*	17-ks. (urinary) mg./day	FSH (urinary)** m.u./day	Sperm counts†	Path No.	Spermatogonia‡	Primary spermatocytes	Secondary spermatocytes	Spermatids	Mature sperm
46.	33	N.	15.0	*+6–18*	10M. 2/hpf	44-2008	+++	+	+	+	+
47.	35	Sm.	N.D.	−30/100 cc.	Zero Zero	42-4278	+++	+	+	+	+
48.	27	N.	10.5	*+52*	1M. Zero	47-7113	+++	+++	+	0	0
49.	25	N.	9.1	*+13–104*	7M. 9M.	44-2517	+++	++	+	+	+
50.	31	N.	18.9	*+13–104* +48–96	60M. 29M.	46-8538	+++	+++	+++	+	+
51.	33	N.	8.3	*+13–104* +48–96	Rare Rare	45-5721	+++	+++	+++	+	+
52.	30.	N.	7.7	*+13–26* −48	Zero Zero	42-5640	+++	++	++	++	0
53.	31	N.	6.5	*+13–52*	Zero Zero	41-6671	+++	++	++	++	0

* N. = normal. Sm. = small. N.D. = not done.

** *See* footnote to Table 3.

† M. = million per cc. Highest and lowest sperm counts are shown in Tables 7, 8, 9 and 13.

‡ The normal for each type of cell described should be +++.

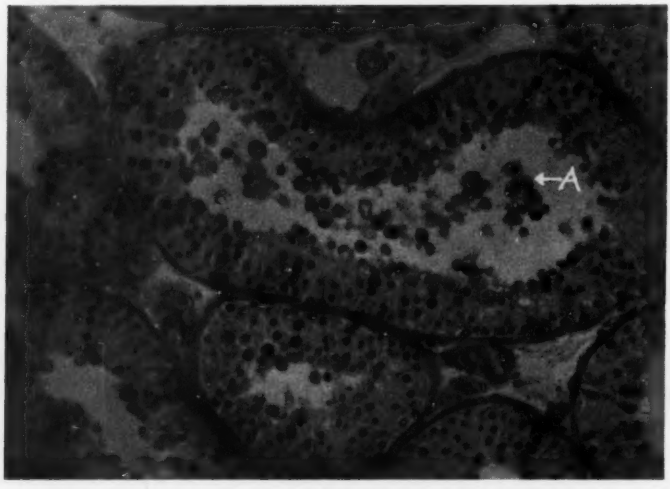

FIG. 9. Case 53. Histologic section of testicular biopsy specimen in a man of 31 years showing arrest of spermatids and no mature sperm. There is slight general reduction of spermatogenic activity. Normal Leydig cells. A = spermatids in the lumen of the tubule ×150.

men had azoospermia or severe oligospermia. The 17-ketosteroid assays and FSH excretions were essentially normal.

The testicular biopsies showed normal Leydig cells, Sertoli cells, and tunica propria. Spermatogonia were present in essentially normal numbers in all tubules but at some point during spermatogenesis there was an abrupt

TABLE 8. GROUP B. CONDITIONS WITH NORMAL FSH EXCRETION

b. WITHOUT ENDOCRINE DISTURBANCE

vii. *Hypospermatogenesis*

Case No.	Age (yrs.)	Size of* testes	17-ks. (urinary) mg./day	FSH (urinary)* m.u./day	Sperm counts*	Path No.	Spermatogenic activity	Mature sperm
54.	26	N.	12.2	*+13–104*	Zero Zero	44-6228	+	0
55.	32	N.	6.4	*+6–26*	Zero Zero	46-6771	+	0
56.	21	Sm.	10.4	*+13–104* +96–192	Zero 2/hpf	46-5671	+	Rare
57.	36	N.	6.2	–96 *+13*	Zero 6/hpf	46-2686	++	0
58.	29	Sm.	10.0	*+13–26*	5/hpf 6/hpf	47-905	++	Rare
59.	32	N.	20.7	*+13–52*	15M. 48M.	47-1743	++	Rare
60.	30	N.	8.5	*+13–104*	2/hpf 16M.	43-6406	++	Rare
61.	28	N.	11.6	*+13–52* –48	25M. 12M.	47-2086	++	Rare
62.	34	Sm.	9.3	*+6*	8/hpf 1/hpf	43-4300	++	Rare
63.	32	N.	10.4	*+13–104*	10M. 32M.	45-4972	++	Few
64.	32	N.	9.8	*+26–52*	2/hpf 2/hpf	43-293	++	Few
65.	23	N.	7.3	*+13–104*	10M. 6M.	45-3356	++	Few
66.	36	N.	6.7	*+13–104*	15M. 10M.	45-1272	++	Few
67.	29	Sm.	16.1	*+13–104* –96	73M. 2/hpf	46-7954	++	Few
68.	31	Sm.	5.3	*+104* *+13–104*	2M. 3/hpf	45-5345	++	Few

* For key to signs and abbreviations, *see* footnotes to Tables 3 and 7.

arrest. This occurred at a remarkably constant stage throughout the tubules of any one testis, but varied from case to case. The arrest occurred at the time of either of the nuclear divisions or at the time of the transformation of spermatids into mature sperm. The early gametogenic cells were desquamated into the lumina, and very few mature sperm were seen (Fig. 9).

This defect in spermatogenesis may be congenital or acquired, nutritional or enzymatic in nature, but is probably not endocrinologic. It is of

interest that 2 patients, Cases 52 and 53, were brothers with identical findings in the biopsy specimens (*see* appendix for further data).

vii. *Hypospermatogenesis*

In Cases 54–68, in contrast to the preceding condition, there was no constant point of arrest but rather a generalized decrease in spermatogenetic activity, *i.e.*, hypospermatogenesis. The data are shown in Table

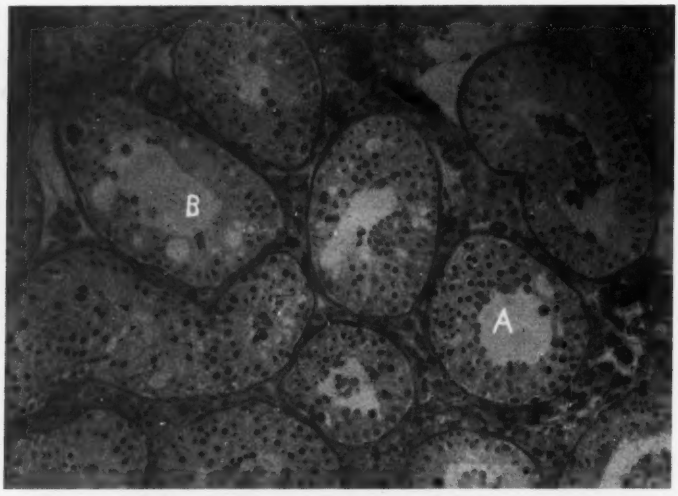

FIG. 10. Case 60. Histologic section of testicular biopsy specimen in a man of 30 years showing hypospermatogenesis of irregular character. The Leydig cells show normal morphology, but in this instance are rather numerous. A = relatively active tubule; B = severe hypospermatogenesis with reduced number of spermatogonia. ×130.

8. These 15 sterile but otherwise normal men had azoospermia or oligospermia with normal assays of urinary FSH and 17-ketosteroids.

As in the preceding condition, the testicular lesion appeared to be confined to the germinal epithelium. Degenerative changes in the Sertoli cells or tunica propria were not conspicuous. The Leydig cells appeared normal morphologically, but in a few instances seemed rather numerous although never aggregated into large masses. The lesion itself varied in degree in different tubules and, in contrast to condition vi., the primary spermatogonia were reduced in numbers (Fig. 10).

This lesion was similar to that found in persons succumbing to a severe illness (43) and presumably to that resulting from hyperpyrexia (44), but such factors were not noted in the histories of the individuals here reported. As the FSH excretions and appearance of the Sertoli cells were

normal we do not suggest an endocrinologic basis for this condition, but regard it as an acquired lesion of the germinal epithelium due to an unknown agent, possibly a nutritional deficiency.

viii. *Oligospermia with normal testicular histology*

Of the 32 cases in subgroup b, there remained 9 men with oligospermia in whom the biopsies showed the testicular tissue to be essentially normal (Table 9). It seems important to emphasize that a decrease in the seminal

TABLE 9. GROUP B. CONDITIONS WITH NORMAL FSH EXCRETION

b. WITHOUT ENDOCRINE DISTURBANCE

viii. *Oligospermia with Normal Testicular Histology*

Case No.	Age (yrs.)	Size of testes*	17-ks. (urinary) mg./day	FSH (urinary)* m u./day	Sperm counts*	Path. No.	Histology of testicular biopsy specimens
69.	34	N.	9.6	–96 *+26*	20M. 6/hpf	44-5230	Variable tubular activity; many sperm
70.	29	N.	14.2	*+13–52* –48	60M. 38M.	47-3393	Variable tubular activity; many sperm
71.	27	Sm.	4.9	*+13–104*	10M. 3/hpf	43-7323	Slight hypospermatogenesis; moderate number of sperm
72.	41	N.	7.5	*+13–104*	70M. 12M.	43-1970	Normal
73	29	Sm.	9.6	*+13–104*	46M. 25M.	44-6754	Normal
74.	33	N.	15.8	+48–96	8M. 22M.	46-7069	Normal
75.	31	N.	10.4	*+6–13*	32M. 15/hpf	47-5339	Normal
76.	39	N.	9.2	*+13–104*	40M.	42-5377	Normal
77.	32	N.	13.2	*+13–104*	30M. 15/hpf	45-5771	Normal

* *See* footnotes to tables 3 and 7 for key to signs and abbreviations.

fluid sperm count with its presumptive implication of male sterility cannot always be correlated with evidence of decreased sperm formation or, for that matter, with any other lesion in the testis. This may be due to our ignorance of some finer detail in testicular histology; or one may assume, as does Charny (5), that the lesion lies in the accessory sexual apparatus.

Group C. Conditions with High FSH Excretion

There were 65 patients (Cases 78–142) in whom the primary lesion appeared to be in the testicular tubules, and in whom, in contrast to Group B, there were secondary disturbances in the endocrine system. In the presence of tubular insufficiency the primary lesion presumably is in the pituitary when the FSH excretion is decreased (*cf.* Group A) and in some element of the testicular tubule when the FSH excretion is increased (*see* diagrams D, E, F, in Figure 18).

Group C is comprised of 7 conditions (ix.–xv.) (Table 2).

ix. *Absence of germ cells*

Cases 78–96 were examples of a condition in which there is complete or almost complete absence of all stages of germ cells. The data are presented in Table 10. Some of these cases were reported in a previous publication (23), and similar ones have recently been well described by del

TABLE 10. GROUP C. CONDITIONS WITH HIGH FSH EXCRETION

ix. *Absence of Germ Cells*

	Case No.	Age (yrs.)	X-ray exposure	Size of testes*	17-ks. (urinary) mg./day	FSH (urinary)* m.u./day	Sperm counts†	Path. No.	Appearance of Sertoli cells	Presence of germ cells	Tunica propria thickening	Peritubular proliferation	Increased mass‡ of Leydig cells‡
(a)	78.	35	No	Sm.	6.2	*+52–104*	Zero	46-3849	N	0	0	0	N
Unknown etiology	79.	36	No	N.	5.9	*+13–52* +192	Zero	46-9355	N	0	0	+	++
	80.	30	No	Sm.	6.4	*+13–104* +96	Zero	47-5337	N	0	0	0	N
	81.	28	No	Sm.	11.3	*+13–104* +96–192	2/hpf	43-2124	N	0	0	0	+++
	82.	24	No	Sm.	11.9	*+13–52* +48–96	Zero 5M.	43-5300	N	one tub.	0	0	+++
	83.	29	No	Sm.	3.0	*+13–104* +192	Zero	41-2139	N	0	0	0	++
	84.	33	No	Sm.	9.2	*+13–104* +96	Zero	43-6487	N	0	0	+	+++
	85.	38	No	Sm.	5.6	*+104* +96–192	Zero	45-3132	N	0	+	+	+++
	86.	28	No	Sm.	5.2	*+52* +96–192	Zero	42-404	N	0	0	0	N
	87.	31	No	Sm.	10.5	*+104 –104*	Zero	40-3879	N	0	+	0	++
	88.	30	No	Sm.	8.8	+96–192	Zero	42-213	N	0	0	0	++
(b)	89.	26	Yes	N.	8.7	*+13–104*	Zero	44-7229	N	0	0	0	N
History of exposure to radioactive agents	90.	25	Yes	N.	9.0	*+13–52* +96–192	Zero	47-6609	N	0	0	0	N
	91.	26	Yes	Sm.	9.4	*+13–104*	Zero	45-2119	N	0	0	0	N
	92.	27	Yes	N.	11.7	*+26–52* +192–384	Zero	42-5062	N	0	0	0	N
	93.	32	Yes	Sm.	14.0	*–206 +104*	2/hpf 2/hpf	42-6311	N	0	++	0	++
	94.	31	Yes	Sm.	11.2	*+104*	2M. 5/hpf	45-1141	N	0	+	0	N
(c)	95.	22	No	Sm.	5.2	*+13–52*	Zero	47-7026	N	0	0	0	+
History of maldescent	96.	18	No	N.	10.2	*–96 +13*	N.D.	47-6743	N	one tub.	0	0	N

* *See* footnotes to Tables 3 and 7 for key to signs and abbreviations.

† Sperm counts were repeatedly zero in nearly all cases.

‡ "Increased mass of Leydig cells" is described from the normal quantity (N), in gradations up to the greatest increase (+++).

Castillo, Trabucco and de la Balze (45). The histologic features have also been recorded by other authors (3, 5, 6).

With the exception of 1 patient, Case 96, who was 18 years old and had large breasts, all were young married adults with normal physical development and potency. However, in the majority, the testes were small.

Of the 18 who had sperm counts, 14 showed azoospermia on each of several examinations. The remaining 4 showed very rare sperm. The level

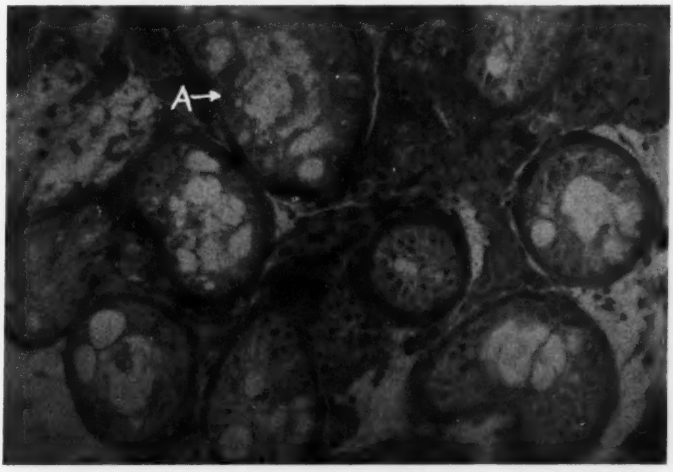

FIG. 11. Case 88. Histologic section of testicular biopsy specimen in a man of 30 years illustrating the absence of germ cells, and normal Sertoli cells. The Leydig cells are morphologically normal, but rather numerous. A = Sertoli cell nucleus. ×170.

of urinary 17-ketosteroids was usually normal, but occasionally rather low. FSH excretions on the whole were slightly high. Few were definitely normal and few were markedly elevated. Thus, out of 13 patients tested by the nondialysis method, 10 showed positive responses at 96 m.u. and only 3 gave a positive response at 192 m.u. per day. This was in contrast to condition x. (*see* following section) in which 22 out of 25 cases showed positive responses, at least on one occasion, at 192 m.u. per day. Absence of testes was also associated with consistently positive FSH assays at 192 m.u. per day.

The testicular biopsy specimens in all 19 cases were almost identical with respect to the tubular lesion. This consisted of medium-sized tubules without any trace of spermatogonia, but with normal-appearing Sertoli cells (Fig. 11). Two cases were exceptional in that a single tubular cross-

section in each biopsy specimen showed not only normal spermatogonia but active sperm formation (Fig. 12). Degenerative changes, *i.e.*, tunica propria thickening and peritubular proliferation, were only rarely encountered, probably no more frequently than might be seen in normal men of the same age range. The Leydig cells were represented by normal types but appeared to be increased in numbers in about one half of the biopsy specimens. That this was an actual increase and not a relative one due to

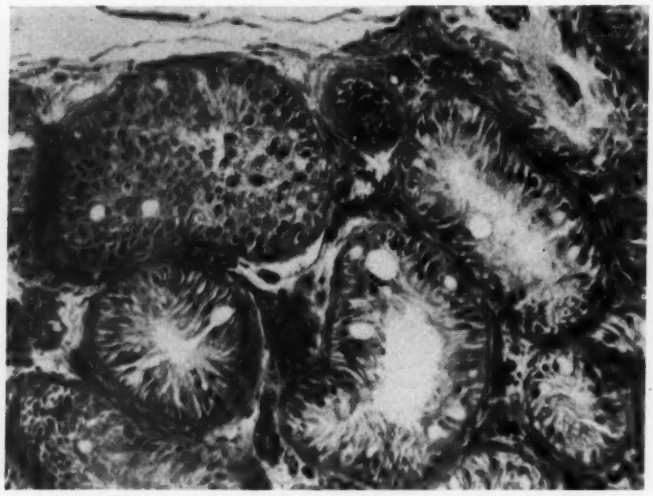

FIG. 12. Case 96. Histologic section of testicular biopsy specimen in a man of 18 years in which the tubules contained no germ cells except in a single instance, as illustrated; this tubule shows normal spermatogenesis with sperm. ×160.

tubular atrophy seemed certain since the tubules were only slightly reduced in diameter.

The etiology of the condition under discussion is not clear. We are not certain that all cases have a common etiology; or, if so, whether the condition should be classified under group C and not group B. It might even be more correct to split the condition into two parts, one for group B and one for group C.

It may be of significance that a history of exposure to radioactive agents was obtained in 6 individuals (Cases 89–94). Of these, 2 received x-ray therapy to the groins, and 4 were in occupations which entailed a radiation hazard. Leroy (46) has published studies on men in Hiroshima and Nagasaki who showed complete destruction of the germinal cells with preservation of the Sertoli cells of the testis, and "castration cells" in the pituitary.

Also of interest are our Cases 95 and 96 with unilateral maldescent. Both patients showed absence of germ cells in the descended testis.

We are impressed with the normal appearance of the Sertoli and the Leydig cells, and we interpret this condition as a selective disorder of the germinal cells with almost normal endocrinologic interrelationships. We are inclined to attribute the tendency to moderately elevated FSH excretions to some minor adjustment in endocrine balance rather than to a fundamental endocrine dysfunction.

The possibility that the germinal epithelium elaborates the "X" hormone, and that the high FSH excretion when it occurs is secondary to the lack of this hormone, comes to mind and is supported by the presence of castration cells in the pituitary found by Leroy (46) in the cases previously cited. Such an explanation would not account for the cases which do not have high FSH excretions or for the failure of the FSH excretion to reach maximal levels in association with complete absence of the germinal epithelium.

The interesting suggestion of Trabucco (47), further elaborated by del Castillo, Trabucco and de la Balze (45), that primordial germ cells may have failed to migrate to the testes is still unproven. It can not explain those cases in which germ cells are found in isolated tubules, or in which a few sperm are found in the semen.

Alternatively, one might hypothesize a functional disorder of the Sertoli cells, not demonstrable by ordinary histologic techniques, which impairs the function of fostering the germinal epithelium more than the other function, producing the "X" hormone.

x. *Sclerosing tubular degeneration*

In Table 11 are presented data on 30 patients (Cases 97–126) who showed clinical evidence of slightly to markedly decreased androgenic function, small but firm scrotal testes, decreased to absent spermatozoa in the semen, a specific testicular histologic picture, elevated urinary FSH excretions, and frequently gynecomastia which first developed during puberty and persisted thereafter. Klinefelter, Reifenstein and Albright (10), who originally reported this condition in 1942 under the title of "Syndrome Characterized by Gynecomastia, Aspermatogenesis without A-Leydigism and Increased Excretion of Follicle-Stimulating Hormone," confined their case reports to patients who had gynecomastia. Since in other respects the patients with gynecomastia differ in no way from those without gynecomastia we, following the lead of Heller and Nelson (11, 12, 48), group them all together. Seven of the original subjects of Klinefelter *et al.* (10) are included here. It is of interest that in Cases 105 and 106 the patients

TABLE 11. GROUP C. CONDITIONS WITH HIGH FSH EXCRETION

x. *Sclerosing Tubular Degeneration*

	Case No.	Age (yrs.)	Gynecomastia*	17-ks. (urinary) mg./day	FSH (urinary)** m.u./day	Path. No	Tubular degeneration	Leydig cell hyperplasia	Leydig cell degeneration
(a)									
With little "eunuchoidism" and with gynecomastia	97.	30	+++	13.1	+205–328	42-3911	Typical	Yes	Slight
	98.	21	+++	7.2	+150–240	42-3601	Typical	Yes	Moderate
	99.	31	+++	10.3	+257–385 +192	38-1941	Typical	Yes	Marked
	100.	37	+	11.7	+384 −192	45-1666	Typical	Yes	Slight
	101.	25	++	3.4	*+13–104* +192–384	46-7737	Typical	Yes	Marked
	102.	17	+++	7.2	+30–50/100 cc. +220–370	42-1912	Typical	Yes	Moderate
	103.	15	++	5.1	+192–384 +96–384	44-1336	Typical	Yes	Marked
	104.	38	++	8.8	*+104* +192	43-7717	Typical	Yes	Moderate
	105.	24	+++	14.7	*+13–26* *+104*	43-6708	Typical	Yes	Moderate
	106.	34 (later)	0 +	13.3	*+26–52* *+104*	43-7522	Typical	Yes	Moderate
	107.	31	++	16.3	+10/100 cc. +384	42-4511	Typical	Yes	Slight
	108.	26	+++	10.1	+96–192 +96–192	43-2119	Typical		Many fibrocytes
	109.	42	++	9.0	+192	45-7636	Typical	Yes	Moderate
	110.	32	+++	9.6	+10/100 cc. +270–450	41-5221	Early	Yes	Moderate
	111.	37	+++	8.6	+261–391 +192	42-3097	Early	Slight	Slight
(b)									
With little "eunuchoidism" and without gynecomastia	112.	32	0	N.D.	+10/100 cc.	42-869	Typical	Yes	Moderate
	113.	19	0	4.9	+192	47-3662	Typical	Yes	Moderate
	114.	29	0	12.0	*+13–104* +384	44-7780	Typical	Yes	Slight
	115.	32	0	9.1	−10/100 cc. +192 later	42-4279	Typical	Yes	Slight
	116.	30	0	N.D.	+10/100 cc.	42-770	Typical	Slight	Moderate
(c)									
With marked "eunuchoidism" and with gynecomastia	117.	45	+	4.7	*+104* +192	42-7417	Typical	Yes	Moderate
	118.	17	+++	1.8?	+100/100 cc. +612–979	41-6084	Typical	Yes	Moderate
	119.	18	+++	4.6	+30–100/100 cc. +50–100/100 cc.	41-7392	Typical	Yes	Marked
	120.	38	+	6.3	*+104* +384	44-2470	Typical	Yes	Moderate
	121.	31	+	6.6	+192 +192	45-3603	Typical		Many fibrocytes
	122.	19	+++	4.0	+576 +384–576	46-7186	Typical		Many fibrocytes
(d)									
With marked "eunuchoidism" and without gynecomastia	123.	22	0	7.0	+50–100/100 cc. +96–192	42-686	Typical	Yes	Moderate
	124.	29	0	3.5	*+52–104* +96–192	46-8734	Typical	Yes	Marked
(e)									
Doubtful examples	125.	14	+++	0.9	*+104*	47-5861	Early		Many fibrocytes
	126.	17	+++	7.0	+106–212	47-2602	Early	Slight	Moderate

* Gynecomastia was described from 0 (absent) in gradations up to +++ (marked). Refer to text for descriptions of "typical" histologic changes.

** For key to interpretation, *see* footnotes to Tables 3 and 7.

were brothers and the man in Case 104 was their uncle. This family has been studied by Reifenstein (49).

The patients with the condition under discussion, at about the usual age, developed an adult phallus and prostate, moderate amounts of axillary and pubic hair, some beard growth, and usually a masculine voice. On the other hand, the typical subject was not as hirsute as the average adult male, although many fell within the normal range. Striking was the lack of chest hair and of abdominal hair in the suprapubic region. This lack of hairiness was the only suggestion of eunuchoidism in 20 of the 28 cases,

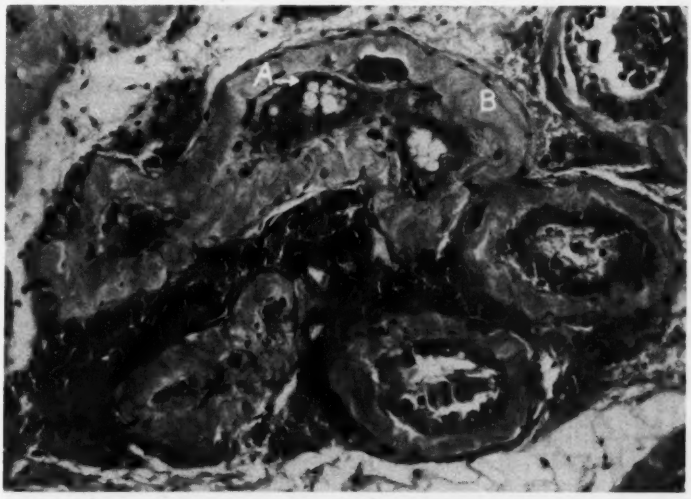

FIG. 13. Case 110. Histologic section of testicular biopsy specimen in a man of 32 years with gynecomastia and elevated urinary FSH. Note the sclerosing tubular degeneration, Sertoli cell atrophy and Leydig cell hyperplasia. A = atrophic Sertoli cell; B = thickened tunica propria. ×164.

but the 8 others showed eunuchoidal skeletal proportions and other evidence of hypoleydigism—few erections, impotency, small prostate gland, small thyroid cartilage, and a feminine voice. Even in these 8 cases the evidence of eunuchoidism was less marked than in typical cases of eunuchoidism-with-low-FSH (Cases 1–5 and 9–19).

Table 11 shows the degree of gynecomastia and urinary assay levels in Cases 97–116, in which there was little evidence of eunuchoidism. The 17-ketosteroid excretion was low in 5 but within the normal range in 13 of the 18 cases tested. The urinary FSH excretion was elevated in all 20 subjects; to be sure, in Cases 105, 106, and 115 the results of the initial tests were normal, but in each of the 3 a high assay level was subsequently obtained.

In Cases 117–124, *i.e.*, the 8 patients with more definite stigmata of eunuchoidism, the 17-ketosteroid excretion was below the normal range. The FSH excretion was markedly elevated in 7 of these 8 cases; indeed, this group included the highest titers we have encountered (*see* Case 118, in which the results were positive for 612 m.u. and negative for 979 m.u. per day).

The analysis of our cases does not confirm the contention of Heller and Nelson (11) that patients falling into this syndrome who have marked eunuchoidism are less likely to have gynecomastia than those with a more normal habitus. Thus, of the 20 cases with little evidence of hypoleydigism, 5 had no gynecomastia, and of the 8 cases with hypoleydigism, 2

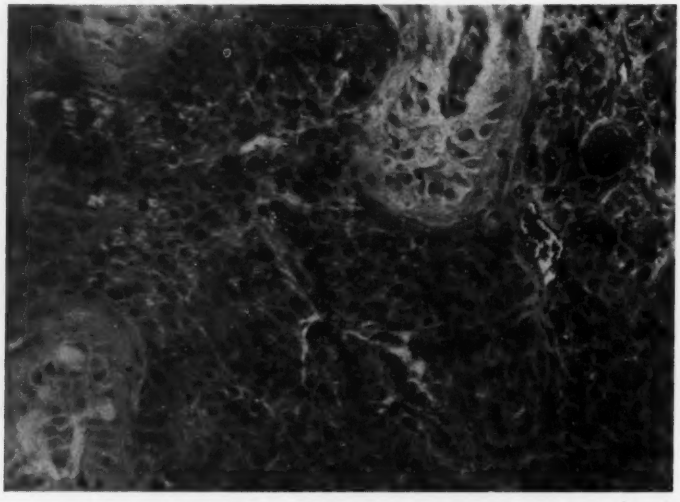

FIG. 14. Case 114. Histologic section of testicular biopsy specimen in a man of 29 years with elevated urinary FSH. Note the marked Leydig cell hyperplasia. ×200.

had no gynecomastia and 3 only slight gynecomastia. Seven of the cases with marked gynecomastia had mastectomies for cosmetic reasons. The breast tissue showed, as previously reported (10), mild ductal stimulation and a more marked periductal fibrous proliferation. This was in contrast to the intense epithelial proliferation and multiplication of ducts which results from estrogen therapy. Further evidence that the gynecomastia is not on the basis of increased estrogen secretion is the finding of normal estrogen excretions in this syndrome (10, 11).

The testicular changes in these 28 cases involved both the tubules and the Leydig cells. The former showed a severe but variable degree of hypo-

spermatogenesis with degeneration of the Sertoli cells. Scattered sperm were found in only a few biopsy specimens. These changes were accompanied by thickening of the tunica propria, the severity of which seemed to be correlated with the degree of Sertoli cell degeneration. Many tubules were completely sclerotic and filled in by the tunica propria (Fig. 13). In approximately one third of these patients infantile tubules were present and in all cases minute sclerotic cords were found that presumably represent the remnants of immature tubules.

The interstitial cells were increased in numbers and often formed masses, which occupied a large proportion of the specimen (Fig. 14). This increment seemed of a different nature than could be accounted for simply by shrinkage of tubules. Furthermore, as pointed out by Nelson and Heller (12), the Leydig cells showed various abnormalities. The histologic details of these changes will be described in detail in a future publication (50). These abnormalities appeared to be of three types: (a) lack of maturation, the interstitial cells being represented by an increase in cells resembling fibrocytes, (b) abnormal cell forms with degeneration, and (c) absence of secretory changes in the cytoplasm.

It was noted that the 6 cases showing the least degree of interstitial-cell aberration had the least evidence of eunuchoidism and had normal 17-ketosteroid excretions (Table 11, Cases 97, 100, 107, 111, 114 and 115). However, no correlation of the histologic findings with the degree of gynecomastia could be made.

Two patients (Cases 125 and 126) were adolescent boys with marked gynecomastia, elevated urinary FSH excretions, low 17-ketosteroid excretion levels, and testes within the normal range for their ages. Histologic sections of the biopsy specimens were not typical, showing only slight degenerative changes of the tubules and relatively normal Leydig cells. The classification of these cases remains in doubt. They may represent an early stage of the condition under discussion.

The explanation here offered for the endocrinologic disturbance in this syndrome (*see* diagram E in Figure 18) is similar to that originally proposed (10). The following 3 abnormalities, it is thought, bear a cause-and-effect relationship to one another in the following sequence: (a) a destructive tubular lesion involving all types of tubular cells but the Sertoli cells in particular, (b) absence of "X" hormone formation to inhibit FSH production by the pituitary, and (c) increased FSH secretion and excretion. From here on, the sequence is more uncertain. The Leydig cell hyperplasia may be secondary to the high level of FSH secretion (*see* previous discussion on the absence of Leydig cells in cases in which the FSH is absent following estrogen administration). The degenerative changes in, and the tendency to hypofunction of, the Leydig cells may be due to lack of "X" hormone to

stimulate LH secretion to stimulate in turn the Leydig cells. Or they may be due to lack of luteotropin, or to any one of several other possibilities. The absence of chest hair and of abdominal hair in the suprapubic area, together with the tendency to scanty beards, somewhat suggests that the androgen production in this syndrome as measured by 17-ketosteroid excretion is derived from the same source as that of the female, namely the adrenal cortex.

There is an alternative interpretation of this syndrome (*see* diagram F in Fig. 18). It is quite possible that the initial abnormality is an inability on the part of the Leydig cells to produce androgen and that lack of androgen gives rise to the tubular degeneration with decrease of "X" hormone production and the other sequelae. The hyperplasia of the Leydig cells could then be explained by increased LH secondary to decreased androgen. Such an explanation would have to include the hypothesis that LH, although increasing the number of Leydig cells, does not correct their inherent defect of not being able to produce androgen. If one accepts this explanation, the question arises as to why 17-ketosteroid production from the adrenal is not compensatorily increased. This and other inconsistencies the authors are unable to answer.

The mammary hyperplasia, it has been suggested (10), may be due to the presence of some testosterone in the face of complete absence of "X" hormone. As an analogy, there is the situation in the castrate dog where Huggins and Clark (51) showed that testosterone stimulates growth of the prostate and that estrogen neutralizes this action. The analogy is all the more pertinent since McCullagh and Walsh (52) showed that "inhibin" caused atrophy of the prostate in intact rats.

The absence of gynecomastia in certain otherwise typical cases of this syndrome and in the 3 cases of mumps orchitis reported subsequently, in which the endocrine balance is very similar, may be due to quantitative differences. It is possible, for example, that in mumps orchitis the "X" hormone production is usually sufficient to prevent gynecomastia but insufficient to inhibit FSH secretion. Lereboullet (53) and Laignel-Lavastine (54) reported a case each in which gynecomastia did occur following a severe bilateral mumps orchitis, though it should be noted that Bénard (55) quotes later evidence that the gynecomastia was not permanent in one of those cases. The failure of gynecomastia to develop in certain cases conceivably might be due to the presence of breast tissue which is refractory to stimulation; this suggestion seems less likely.

The development of gynecomastia in certain eunuchoid patients during testosterone therapy supports the above explanation. On the other hand, against this thesis is the gynecomastia occurring after prepubertal castration in some of the men studied by Wagenseil (56, 57).

xi. *Absence of testes*

In Table 12 are summarized 4 cases in which surgical explorations revealed either no testicular tissue or at most rudimentary tissue. Thus they were virtually prepubertal castrates. In Case 130 the patient had developed bilateral swellings of the gonads at the time of birth, but in the other cases no cause for the condition was found. In 3 of these 4 cases, surgical exploration had been so thorough that it is doubtful that a cryptorchid

TABLE 12. GROUP C. CONDITIONS WITH HIGH FSH EXCRETION

xi. *Absence of Testes*

Case No.	Age (yrs.)	Exploratory findings and endocrine therapy	Pubic hair	Adult phallus	Breasts	17-ks. (urinary) mg./day	FSH (urinary) m.u./day	FSH (urinary) m.u./100 cc.
127.	6	Rudimentary scrotal testes						
	18	No endocrine therapy	+	No	Fat	N.D.*	N.D.	
	24	Testosterone propionate from 22 to 24 yrs.						
	26	No endocrine therapy	++	Yes		18.0	+576	
128.	18	No endocrine therapy	+	No	0	N.D.		N.D.
	23	APL & testosterone therapy from 22 to 23 yrs.						
	25	No endocrine therapy	++	Yes		4.9		+10
	26	Right canal & abdomen; negative findings						
		Left canal showed tiny body						
129.	20	No endocrine therapy	+	No	0	2.4		+30
	20	Right vas in scrotum; no testis						
		Left side not explored						
130.	12	Abdominal explor.: negative results						
	16	No endocrine therapy	++	Yes	0	N.D.		N.D.
	18	APL therapy						
	19	No endocrine therapy	++	Yes	Fat	8.8		+30
	21	Scrotal explor.: negative results						
		Breast biopsy specimen showed fat						

* N.D. = not done.

organ could have been overlooked; in the fourth (Case 129) one vas was located but this did not terminate in glandular tissue.

On physical examination these 4 youths differed surprisingly little from the typical case of eunuchoidism-with-low-FSH. Thus, they had eunuchoid proportions, high voices with small thyroid cartilages, small prostates, and the other signs of hypoleydigism. The axillary and pubic hair, on the other hand, tended to be more abundant, approaching that of the normal female, and in Case 130 the patient showed a moderately well-developed phallus. These 4 youths did not have gynecomastia.

The urinary 17-ketosteroids in the 4 cases varied from high (18.0 mg.

in Case 127) to low (2.4 mg. in Case 129). On theoretic grounds (*see* subsequent section) the authors feel that as this group becomes larger it will be found that the 17-ketosteroid excretions tend to be as high or higher than those of normal females.

Our interpretation of the disordered endocrine pattern in these cases with life-long absence of testes is shown in diagram D, Fig. 18. The absence of the inhibitory action of Sertoli-cell hormone permits excessive FSH secretion. The eunuchoidal habitus is attributed to the lack of functioning Leydig cells. Since the adrenal cortex is not involved, one would expect that the 17-ketosteroid excretion would be at least equal to that of the normal female; indeed, there is reason to believe it might be even higher since Woolley (58, 59) found that gonadectomy in very young mice leads to tumors of the adrenal cortices. Whether LH is high or low in this syndrome may depend on which of the following two factors predominates: (a) the lack of Leydig cell hormone to inhibit LH, and (b) the lack of "X" hormone to stimulate LH. On the answer to this question would depend the production of the adrenal cortical "N" hormone, if the latter really is stimulated by LH. As both Sertoli-cell hormone and Leydig-cell hormone are absent, gynecomastia fails to develop (10).

Heller, Nelson and Roth reported 6 similar cases under the title of "Functional Prepuberal Castration in Males" (9). Their findings differed from ours in that 3 of their cases had gynecomastia and in that the 17-ketosteroid excretions tended to be lower than in the group which they call "tall eunuchoids with low gonadotropins" and which we call "eunuchoidism-with-low-FSH."

xii. *Normal testicular histology (? climacteric)*

In Cases 131–136 the patients showed elevated FSH excretions but normal testicular histology in the biopsy specimens. This condition is labelled as the "male climateric." The data are summarized in Table 13.

The chief clinical manifestation of these men was impotence in the 3 over 50 years old and oligospermia with presumptive sterility in the younger men. One man, Case 132, had nervous symptoms and flashes as seen in menopausal women. There were no abnormalities of development and no gynecomastia. The urinary 17-ketosteroids tended to be low and the FSH excretions were, of course, high.

The testes showed active spermatogenesis and apparently normal Sertoli and Leydig cells. Sclerotic tubules were not conspicuous.

These cases differed from those in condition x. (sclerosing tubular degeneration), in that they did not show the typical testicular changes, and from those in condition viii. (oligospermia with normal testicular histology), in that they showed unquestionably high FSH excretions.

It is our practice to divide the climacteric in both sexes into two stages: (a) compensated, and (b) decompensated. In the compensated stage there is a tendency to decreased production of one of the gonadal hormones (estrogen in the female and "X" hormone in the male); this tendency is met by overproduction of FSH, so that the gonadal function remains essentially intact. In the decompensated stage this tendency to gonadal hormone failure is not counterbalanced in spite of increased production of FSH, and gonadal failure becomes clinically demonstrable. In the female

TABLE 13. GROUP C. CONDITIONS WITH HIGH FSH EXCRETION

xii. *Normal Testicular Histology (?Climacteric)*

Case No.	Age (yrs.)	Size of testes*	Flashes, nervousness	Impotence	"Sterility"	17-ks. (urinary) mg./day	FSH (urinary)* m.u./day	Sperm counts*	Path. No.	Sertoli cells*	Spermatogenesis	Leydig cells
131.	75	N.	0	Yes	No	3.9	+192	N.D.	45-1796	N.	N.	N.
132.	55	N.	Yes	Yes	No	4.7	+192 +96–192	N.D.	44-6747	N.	N.	N.
133.	50	N.	0	Yes	?	10.2	+192	N.D.	45-180	N.	N.	N.
134.	37	Sm.	0	No	Yes	5.7	*+104*	24M.	45-817	N.	N.	N.
135.	32	N.	0	No	Yes	9.8	+192	6M. 15M.	45-6338	N.	N.	N.
136.	30	N.	0	No	Yes	7.0	+192	1M. 69M.	43-4003	N.	N.	N.

* For key to signs and abbreviations, *see* footnotes to Tables 3, 7 and 12.

the compensated stage lasts a very short time; in the male, on the other hand, one seldom meets the decompensated stage. In the compensated stage in the male, therefore, one would anticipate that the Sertoli cells would be normal microscopically, as indeed they appeared to be.

Nelson (16, 17) classified as the male climacteric, cases with similar assay findings and essentially normal seminiferous tubules, but decreased numbers of Leydig cells with abnormal cytologic characteristics. However, we have been unable to recognize such Leydig-cell defects in our 6 biopsy specimens.

xiii., xiv., and xv. *Miscellaneous conditions, and one unclassified case*

The relevant details of the last 6 cases are summarized in Table 14.

Three patients, Cases 137–139, had suffered in the past from bilateral mumps orchitis. All were normally developed and potent, but sterile. Two had small testes, but the third (Case 139), who had had orchitis twelve months before, had testes of normal size at the time of study. The urinary 17-ketosteroids were normal in the 2 younger men, but very low in Case

138, a 50-year-old man, who also had lymphatic leukemia (*see* Appendix). In each case the urinary FSH excretion was elevated.

The testes showed gradations from focal to generalized tubular sclerosis, and irregularly distributed but normal-appearing Leydig cells in medium to small groups in a dense collagenous interstitial tissue (Fig. 15).

TABLE 14. GROUP C. CONDITIONS WITH HIGH FSH EXCRETION

xiii., xiv., xv. *Miscellaneous Conditions*

Diagnosis	Case No.	Age (yrs.)	Size of testes	17-ks. (urinary) mg./day	FSH (urinary)* m.u./day	Sperm counts*	Path. No.	Description of testes
Mumps orchitis at 15 yrs. (bilateral)	137.	31	Sm.	8.9	*+104*	Zero	43-6966	Complete tubular sclerosis: interstitial fibrosis: normal Leydig cells.
Mumps orchitis at 25 yrs. (bilateral)	138.	50	Sm.	2.0	+192	N.D.	48-967	Focal areas showed normal tubules and many normal Leydig cells; elsewhere complete tubular sclerosis, interstitial fibrosis and small groups of normal Leydig cells.
Mumps orchitis at 30 yrs. (bilateral)	139.	31	N.	8.9	*+104* +192	17M. 1/hpf	47-6611	Focal areas of tubular sclerosis and interstitial fibrosis; elsewhere tubules showed variable activity and rather numerous but normal Leydig cells.
Myotonic dystrophy	140.	63	Sm.	3.5	*−6*	N.D.	47-4097	Complete tubular sclerosis; no interstitial fibrosis; normal Leydig cells.
Cryptorchidism (bilateral)	141.	17	0	6.0	+192	N.D.	48-1762	Rare degenerating germ cells; slight atrophy of Sertoli cells; small groups of foamy Leydig cells.
Tubular sclerosis undiagnosed	142.	47	Sm.	8.5	+170	N.D.	43-608	Complete tubular sclerosis; no interstitial fibrosis; normal Leydig cells.

* For key to signs, and abbreviations, *see* footnotes to Tables 3, 7 and 12.

This picture differs from that found in condition x. (sclerosing tubular degeneration) in which there is less interstitial collagen and more abnormalities of the Leydig cells.

It will be noted that the hormonal pattern in bilateral orchitis following mumps is apparently similar to that in the syndrome described as condition x. Thus both have high FSH excretions and relatively normal 17-ketosteroid excretions. Why both conditions are not invariably accompanied by gynecomastia has already been discussed (*see* discussion under condition x.).

Case 140, a man of 63 years, had myotonic dystrophy and bilateral tes-

ticular atrophy. The testes showed complete tubular sclerosis without excessive interstitial fibrous tissue and with normal Leydig cells. It was noteworthy that despite the decreased testicular volume there was no condensation of Leydig cells to simulate hyperplasia. A high urinary FSH level had been expected in this case but the assay was less than 6.5 m.u.

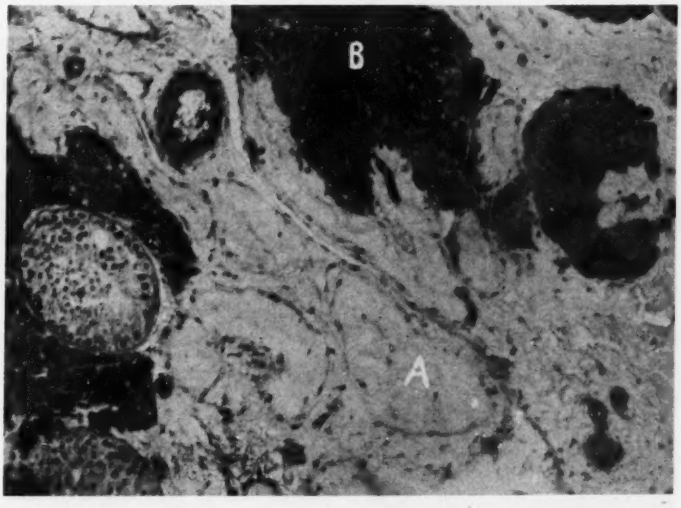

FIG. 15. Case 138. Histologic section of testicular biopsy specimen in a man of 50 years, showing the result of mumps orchitis, *viz.*, widespread tubular sclerosis, interstitial fibrosis, a few normal tubules and scattered groups of normal Leydig cells. (The prominence of the interstitial fibrous tissue rather than of the Leydig cells is better illustrated in Figure 1 of another paper (23), showing a section from Case 136.) A = sclerotic tubule; B = large group of Leydig cells. ×160.

per day. This might be due to the severe debilitation in this man. Klinefelter (60) found a high FSH excretion in 1 case of testicular atrophy associated with myotonic dystrophy. Benda and Bixby (61) described similar biopsy pictures in 2 cases of this condition, and in 1 of them the urinary FSH level was assayed and found elevated. It thus appears that the tubular sclerosis, which involves the Sertoli cells, may result in elevated FSH secretion in this condition. The absence of Leydig-cell hyperplasia serves to differentiate this syndrome from that described as condition X.

Case 141 was a short 17-year-old cryptorchid youth. His phallus was adult-sized but he had scant pubic hair, no beard and a high voice. The urinary 17-ketosteroids were normal but the FSH was elevated. The initial clinical impression was that he had no testicular tissue (*cf.* condition

xi.) and an associated dwarfism. However exploration revealed bilateral abdominal testes with a characteristic histologic picture (Fig. 16). This consisted of small tubules with thick tunica propria, degenerating spermatogonia and persistent Sertoli cells showing atrophic changes. The Leydig cells were in small groups and their cytoplasm was abnormally foamy. This case is probably an example of failure of the testes to descend because of

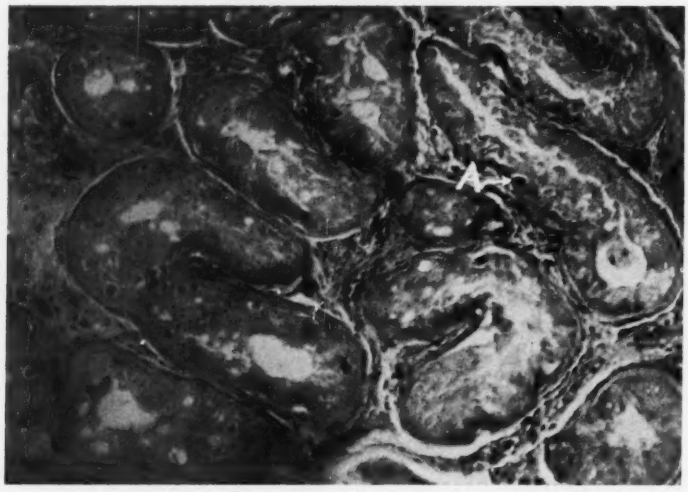

FIG. 16. Case 141. Histologic section of testicular biopsy specimen in a man of 17 years with bilateral undescended testes. Only a few inactive spermatogonia remain and the cytoplasm of the Sertoli cells is somewhat atrophic. A = spermatogonium. ×150.

some mechanical, embryologic defect; the endocrine manifestations are secondary to testicular changes resulting from the abnormal environment, notably the increased heat of the abdomen as compared with that of the scrotum. The elevated FSH excretion, we presume, is due to a defect in the production of the Sertoli-cell hormone.

It must not be concluded that all cases of cryptorchidism would give the same picture. In some cases the primary factor may be endocrinologic, such as hyposecretion of pituitary gonadotropins. For example, a 23-year-old male (M. G. H. #5319) had a typically eunuchoid habitus and urinary FSH assays were less than 6.5 m.u. per day. Testicular descent followed endocrine therapy.

Case 142 has proven difficult to classify. This 47-year-old man complained of impotence of recent onset. He had eunuchoidal traits, moderate gynecomastia and very small scrotal testes. Urinary 17-ketosteroids averaged 8.5 mg. per day, and the FSH excretion by the non-dialysis method

was greater than 170 m.u. per day. The testicular biopsy showed complete tubular fibrosis without interstitial fibrous tissue proliferation, with the Leydig cells present in groups of normal size, some of the cells showing degenerative changes. This case may represent a variant of condition x. (sclerosing tubular degeneration) in which the expected Leydig-cell hyperplasia was absent for some unknown reason, possibly the patient's age (12, 13). On the other hand, it may represent an example of the climacteric in the "decompensated stage."

Comparative Diagrams

The endocrinologic interpretations which have been suggested are represented diagrammatically in Figures 17 and 18.

DISCUSSION

FSH test, a measure of FSH and not of FSH+LH?

Heller (62), with considerable justification, objects to the interpretation we place upon the follicle-stimulating hormone (FSH) test, on the ground that it measures LH as well as FSH. He prefers to speak of it as a test for gonadotropins.

In the first place, he calls attention to the observation of Greep, Van Dyke and Chow (63) that in the hypophysectomized rat pure FSH will not cause estrogen secretion and hence uterine enlargement without the addition of a small amount of LH. This phenomenon might lead to a false negative response to the FSH test.

We believe that the minute amount of LH necessary for the ovarian follicles to produce estrogen may be already present in the infantile mouse used in the FSH test. According to Simpson (64), in the infantile rat there are present rounded masses of interstitial cells; after hypophysectomy these interstitial cells shrink, their nuclei become pyknotic, and the cells lose their epithelial characteristics. This strongly suggests that the infantile rat is producing some LH. If the same phenomenon occurs in the infantile mouse, it would appear that enough LH may be present to allow FSH to produce estrogen.[2]

In the second place, Heller and Nelson (21) call attention to the well known augmentation in the enlargement of the ovaries which occurs

[2] Dr. R. O. Greep was kind enough to carry out experiments using the ventral prostate method in hypophysectomized rats. He demonstrated the presence of some LH in aliquots of the alcoholic urinary extracts prepared for the FSH tests in two of the patients which he tested. Thus, LH apparently is present in at least small amounts in some if not all of our extracts.

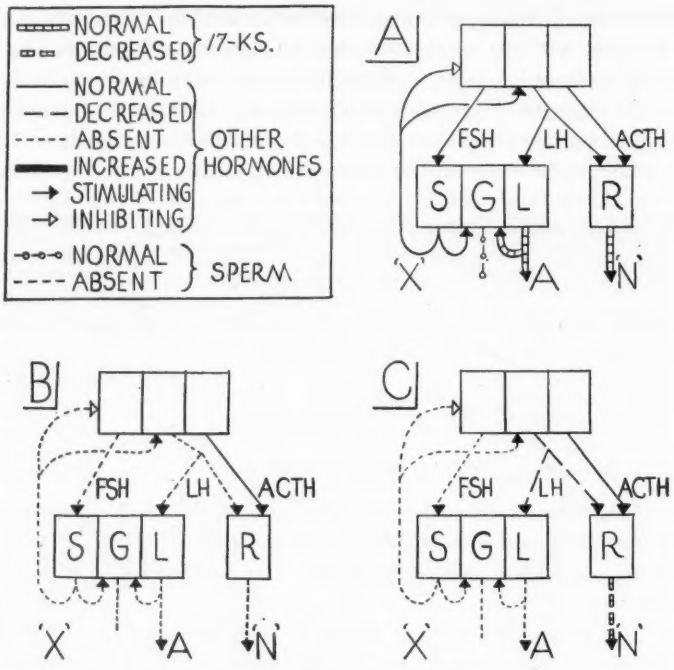

FIG. 17. *Schematic diagram designed to contrast normal hormonal pattern in males with hypogonadism secondary to various deficiencies of pituitary gonadotropins.*

A. Normal adult (*see* Fig. 1).

B. Normal prepubertal boy or eunuchoidism-with-low-FSH-and-LH (*see* Cases 6–8). For explanation of diagram, *see* Figure 1.

It should be noted that this diagram differs from that which one would draw for panhypopituitarism, in that the production of ACTH is represented as normal. It should be further noted that the reticular zone (R) of the adrenal cortex is represented as producing no "N" hormone on the assumption (*see* text) that both ACTH and LH are necessary for the production of this hormone.

C. Eunuchoidism-with-low-FSH (*see* Cases 1–5 and 9–19).

The primary disorder is thought to be absence of FSH production. In addition there are absence of Leydig cells and low but not absent 17-ketosteroid excretion. In this diagram these latter are represented as due to lack of "X" hormone to stimulate LH, but there are other possibilities (*see* text).

when LH is added to FSH, and point out that this phenomenon might lead to a falsely positive response to the FSH test.

Although this augmentation factor is important in the test as carried out by Heller *et al.* (65, 66), it may not be in the method used in this laboratory (29). Whereas they measure the units of gonadotropin by the degree to which the ovaries and uterus are enlarged, we depend on assays

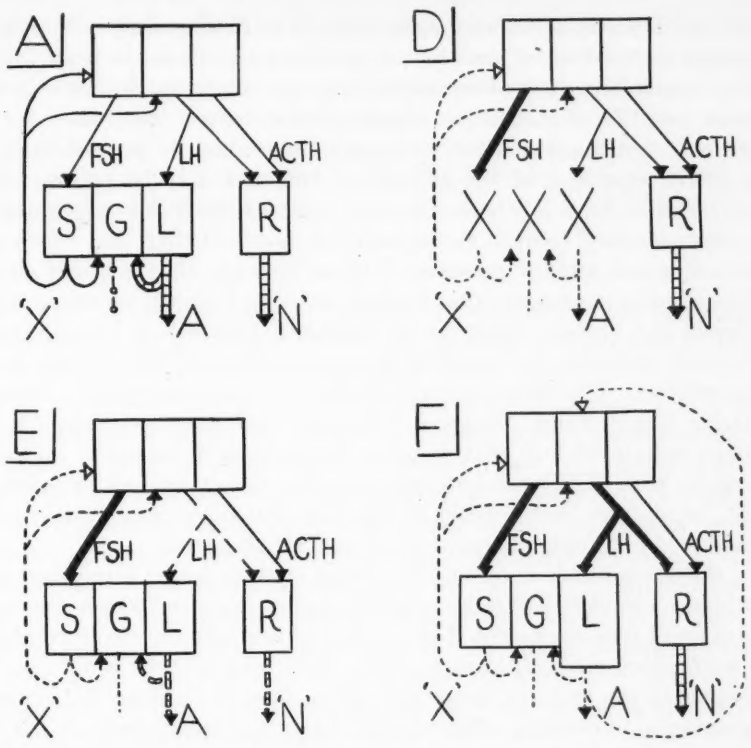

FIG. 18. *Schematic diagrams designed to contrast normal hormonal pattern in males with certain testicular deficiencies associated with high FSH excretions*

A. Normal adult male (*see* Fig. 1).

D. Absence of testes (*see* Cases 130–133).

All functions of the testis are absent. Lack of "X" hormone, in addition to causing increased FSH, leads to decreased LH; lack of Leydig-cell hormone leads to increased LH. Whether LH is increased or not may depend on which of these predominates.

E. Sclerosing tubular degeneration (*see* Cases 97–126); first interpretation.

The primary lesion, according to this interpretation, is a destructive lesion of tubular elements. This leads to lack of "X" hormone, with resulting increased FSH. The decreased androgen production is secondary to decreased LH, which in turn is due to lack of "X" hormone to stimulate LH.

F. Sclerosing tubular degeneration; alternative interpretation.

The primary lesion, according to this interpretation, is an inability of the Leydig cells to produce hormone. Lack of Leydig-cell hormone results in tubular sclerosis, lack of "X" hormone and increased FSH. Furthermore, lack of Leydig-cell hormone allows an increased production of LH with resulting increase in numbers of Leydig cells, albeit still inherently defective in function.

The enlargement of the Leydig cell (L) box is to emphasize the increased numbers of Leydig cells. In contrast to other diagrams, the inhibiting action of Leydig-cell hormone on LH is indicated by an inhibiting arrow.

at various dilutions to determine the units of FSH, the result at any level depending on whether or not there is present a significant increase in the uterine weight. Since LH alone has no effect on follicle development in the infantile rat (67) or mouse (*see* theory of Hamburger (68)), in order to obtain this significant increase in weight there must be present at least some FSH, regardless of the amount of LH that may be present. The question comes down to whether it would take less FSH to produce significant uterine enlargement in the presence of more LH than that which the immature mouse already possesses. The authors feel that it might not.

Thus, since it is probable that the follicle in the infantile mouse must be developed to a certain degree by administered FSH before LH can cause further enlargement, it is possible that this amount of FSH would be already sufficient to produce enough estrogen to cause a significant increase in uterine weight. Evans, Simpson, Tolksdorf and Jensen (69) showed in the hypophysectomized rat that human chorionic gonadotropin, although synergistic with FSH when both were injected simultaneously in sufficient quantities, did not lead to follicular development when combined with 0.5 rat unit (r.u.) of FSH, whereas 1.0 r.u. of FSH alone did.

On the other hand, Simpson (70) found that, in intact immature rats, five times more FSH had to be administered to produce 100 per cent augmentation of ovarian weight if given alone, than when given in combination with chorionic gonadotropin. This latter observation is not directly applicable to the problem under discussion, since it concerns 100 per cent augmentation of ovarian weight rather than a significant increase in uterine weight. Nor are those observations of Evans *et al.* (69) directly applicable, in which they show that 1.0 r.u. of FSH is augmented by 2.0 r.u. of ICSH (LH). The problem at issue is whether any amount of ICSH will lead to uterine enlargement when injected simultaneously with less than 1.0 m.u. of FSH.

It should be pointed out that augmentation of FSH by chorionic gonadotropin is not the same as augmentation by LH. Chorionic gonadotropin apparently can stimulate a more immature follicle than can LH, since pure LH has no effect on the infantile rat ovary, whereas chorionic gonadotropin does (*cf.* pregnancy test).

Furthermore, almost all the work on augmentation applies to the ovaries. To be sure, Evans *et al.* (69) record the uterine sizes and they do show some augmentation, but less definitely so than in the case of the ovaries. For example, 2.0 r.u. of ICSH (LH) produced no uterine enlargement when added to 1.0 r.u. of FSH, but 4.0 and 8.0 r.u. of LH definitely did ((69), Table 6).

From the above discussion the authors conclude that the criticism that the FSH test as carried out in this laboratory is a measure of FSH plus LH has not been proved or disproved. To call the substance measured "gonado-

tropins" if it is only FSH, is almost as erroneous as to call it "FSH" if it is both FSH and LH. The question could be answered by determining how much pure FSH constitutes 1.0 immature-nonhypophysectomized-mouse-unit with respect to uterine enlargement, and then determining whether 0.75 and 0.50 of this unit will produce the same effect on the uterine weight when combined with an excess of LH. The difficulty is in obtaining pure preparations of FSH and LH.

The nature of gonadotropins in urine

Whereas, as just discussed, we believe that the FSH test as performed in this laboratory may measure FSH alone, this does not mean that LH may not be also present in normal or abnormal conditions.

Only small amounts of gonadotropins, if any, are present in the urine of prepubertal boys (66, 71, 72). Simpson (64), using hypophysectomized rats, found both FSH and ICSH (LH) to be present in the urine of normal adult males, the latter predominating slightly. That LH does appear in increased amounts in the urine under certain conditions has been demonstrated by a different method by Heller, Nelson and Roth (9) and by Heller and Nelson (11), and similar findings were reported by Goldzieher and Hamblen (73). Certainly a reliable clinical test for LH alone in the blood or urine would be a great help in sorting out the various disorders. The application to the above problem of the ovarian hyperemia reaction in mice, as developed by Lloyd and his co-workers (74), may be an important step towards clarifying this matter.

Is high urinary excretion of FSH an indication of increased production by the pituitary or decreased utilization by the gonads?

Heller, Heller and Sevringhaus (75) advanced the hypothesis that the high FSH excretion in both menopausal and ovariectomized women is due to a failure of the ovaries to remove FSH from the blood. The same reasoning was carried over to males, in this case the rise in FSH excretion being attributed to a failure of the seminiferous tubules to utilize it (11, 76). Such an interpretation does not explain the increased gonadotropin content of the pituitary of castrated animals; it rather implies that most of the blood must go through the ovaries before it goes through the kidneys; and, most of all, it in no way explains the decreased FSH excretion that follows estrogen administration. The authors prefer the explanation that FSH stimulates production of estrogen in the female, and of "X" hormone in the male; that both these hormones in turn inhibit the production of FSH; and that, therefore, in the absence of the gonads there is an overproduction of FSH.

Evidence for a second testicular hormone

In this clinic attention first became focused on the possibility of there being a second testicular hormone, the "X" hormone,[3] when we became interested in the syndrome described by Klinefelter, Reifenstein and Albright (10) (*see* condition x.). It seemed quite clear that in this syndrome nature had destroyed one component of the testis (the tubules) and had left intact the other (the Leydig cells). It also seemed clear that the increase of the excretion of FSH in this condition must be due to the loss of an FSH-inhibiting hormone derived from the tubules and not to the loss of the hormone produced by the Leydig cells, presumably testosterone. To be sure, it might be argued, as have Nelson and Heller (11, 12), that the Leydig cells, though plentiful in this condition, are abnormal in histologic appearance and deficient in function, as suggested by a tendency to incomplete virilization in patients with this syndrome. This objection can be met, however, by the findings in patients with bilateral mumps orchitis where one encounters destruction of the tubules and an overproduction of FSH coupled with normal-appearing Leydig cells, normal virilization, and normal excretion of 17-ketosteroids.

Further clinical evidence for the presence of the "X" hormone is derived from the fact that it requires much larger doses of testosterone to lower the FSH excretion than to restore the secondary sex characteristics (9, 14, 19, 22, 48, 78, 79, 80). Figure 19 shows a study on the patient in Case 130, in which large doses of testosterone failed to lower the FSH excretion whereas moderate doses of diethylstilbestrol did. The effect of estrogens on lowering the FSH excretion has also been observed by others (19, 66, 80) and was likewise demonstrated in Cases 33, 34, and 35 in the present study.

Heller and Nelson (11), the chief proponents of the single testicular hormone theory, point out that the second hormone has not been isolated, and also (48) that testosterone in sufficiently large amounts will depress the FSH excretion.

The presence of a second or "X" hormone from the testis is of course no new concept. Following up the work of Mottram and Cramer (81) and Martins and Rocha (82), McCullagh (77) and McCullagh and Walsh (52) described a nonandrogenic water-soluble testicular hormone, which they called "inhibin." Among other properties, "inhibin" caused atrophy of the prostate and seminal vesicles of normal rats. Törnblom (83) found in castrate rats that doses of testosterone sufficient to restore the weights of

[3] For the second testicular hormone we use the term "X" hormone instead of "inhibin" introduced by McCullagh (77), because the connotation of being water-soluble goes along with the latter; whether the second hormone is water-soluble or not we leave entirely open.

prostate and seminal vesicles did not alter the pituitary hypertrophy characteristic of castrate animals. This latter observation was confirmed by McCullagh, Schneider, Bowman and Smith (84) who also reviewed the evidence for a second testicular hormone.

Nature of "X" hormone

It is not proposed to review the reasons for and against the suggestion that "X" hormone is an estrogen. The evidence has been reviewed recently by Huggins and Moulder (85). Marked tubular damage is produced by estrogen administration to men (*see* Cases 33–37), but in such instances the quantity given is presumably much greater than the amount in which the naturally occurring "inhibin" would be secreted.

There is some evidence that this hypothetic hormone is Δ^5-pregnenolone which has been isolated from swine testes by Ruzicka and Prelog (86). Selye (87, 88) demonstrated in hypophysectomized rats a beneficial effect from this steroid on spermatogenesis without a concomitant repair of the atrophy of the Leydig cells or of the prostate. Albert and Selye (89) showed that this steroid prevented the testicular atrophy occurring in estradiol-treated rats.

Sertoli cells as a source of the "X" hormone

From the above discussion it has been concluded that the tubules produce a second or "X" hormone which inhibits FSH secretion. The next question is whether the "X" hormone is produced by the Sertoli cells or the gametogenic cells. The following considerations suggest that it is produced by the Sertoli cells:

1. The "X" hormone is the analogue in the testis of estrogen in the ovary; estrogen is produced by the granulosa or theca cells and not by the female gametocytes (*i.e.*, the ova): hence, "X" hormone is probably produced by the Sertoli cells and not by the male gametocytes (*i.e.*, the germ cells).
2. The Sertoli cells show maturation at the time of puberty so that from the point of view of this time relationship they are at least as likely as the gametocytes to be the source of "X" hormone formation.
3. The cytologic characteristics of the Sertoli cells—contiguous with basement membrane and hence not far removed from blood supply—are compatible with those of other cells of internal secretion.
4. As Huggins and Moulder (85) have pointed out, the Sertoli cells, like so many steroid-producing cells (*e.g.*, Leydig cells), contain lipoid; the same authors also showed that Sertoli-cell tumors in dogs were hormone producing.
5. In certain patients (Cases 46–96), in whose testes the demonstrable tubular lesion is confined to the germ cells, there is seldom symptomatic evidence of endocrine disorder and the FSH excretion is normal (Cases 46–77), or only moderately elevated (Cases 78–96) (*cf.* discussion under condition ix.); in certain other patients (Cases 97–130 and 137–142), by comparison, in whose testes the lesion includes the Sertoli cells as well as the gametocytes, the FSH excretion is markedly elevated.

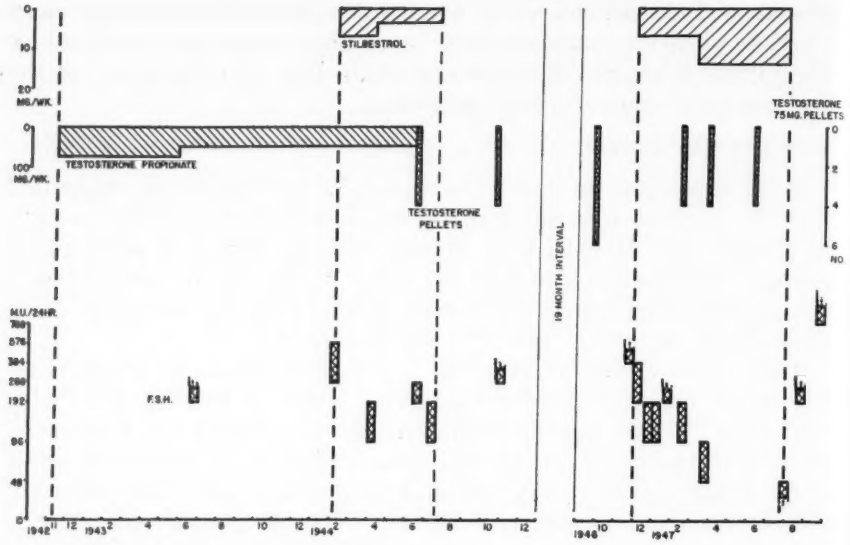

FIG. 19. *Study contrasting the effects of androgen and estrogen on the excretion of FSH in the urine in a patient with no testes (Case 130).*

Androgen was injected in the form of testosterone propionate (25 mg. two or three times a week) or implanted in the form of testosterone pellets (4 or 6, 75-milligram pellets every two to four months). Testosterone pellets can be considered effective for three to four months. Estrogen was administered orally in the form of diethylstilbestrol (0.5 to 2.0 mg. daily).

Note that the diethylstilbestrol reduced the FSH excretions much more effectively than did either of the androgenic preparations.

Note the changing scale for FSH excretion. The level of the latter is represented by a column, the lower end of which delineates the highest positive point above which the assays were found negative, and the upper end of which delineates the lowest negative point below which the assays were found positive. A broken end at the top of a column indicates that the upper limit of excretion was not determined; a broken end at the bottom indicates that the lower limit of excretion was not determined.

Actions of the "X" hormone

The "X" hormone is an essential factor for the promotion of spermatogenesis. Secondly, it inhibits the hypophyseal secretion of FSH. Thirdly, it may stimulate the hypophysis to produce LH (or possibly luteotropin). Such an effect has been attributed to its ovarian counterpart, estrogen (37, 38, 90, 91, 92, 93, 94, 95). It is the loss of this action of the Sertoli cells which we have invoked in our first explanation of the diminished function and abnormal appearance of the Leydig cells in the syndrome with sclerosing tubular degeneration. Similarly, if one grants this property of the "X"

hormone, one could explain the whole picture in idiopathic "eunuchoidism-with-low-FSH" on the basis of the FSH lack, the sequence being 1) FSH lack, 2) "X" hormone lack, 3) failure of stimulation of LH, and 4) absence of Leydig cells. Finally, as discussed previously (*see* condition x.), the "X" hormone may have as a fourth factor an inhibitory effect on the development of gynecomastia.

Evidence against the third action of the "X" hormone—to stimulate LH production—is the presence of normal Leydig-cell function in most patients with bilateral testicular atrophy following mumps, although, as mentioned before, feminization occurs in isolated cases (53, 54) of this condition.

The authors have been struck by the dearth of abnormal clinical findings in patients who lack the "X" hormone. Thus a patient with bilateral testicular atrophy following mumps and with a high titer of FSH, characteristically has normal potency, normal libido, normal 17-ketosteroid excretion, no premature aging, no osteoporosis, and no gynecomastia. This is in contrast to a female patient with absence of estrogen.

Relationship between FSH excretion and the number of Leydig cells

One of the characteristics of the conditions with low FSH excretion (*see* Cases 1–37) is that the Leydig cells are either absent, or rare and degenerating. This is particularly noteworthy when FSH is depressed by estrogen therapy (*see* Cases 33–37).

Furthermore, conditions associated with normal FSH excretion, with few exceptions, show normal numbers of Leydig cells. Moreover, in certain conditions with high FSH excretions (*e.g.*, sclerosing tubular degeneration, condition x.) there is frequently an increase in the Leydig cells, whereas in others (*e.g.*, mumps orchitis), there is no such increase.

Therefore, in general, there is a parallelism between the level of FSH excretion and the numbers of Leydig cells; but there is no constant relationship between the level of FSH excretion and the Leydig-cell function as indicated by cytologic characteristics, 17-ketosteroid excretion or clinical signs of androgen activity (*see* condition x.).

These facts by themselves might suggest that the FSH test as here performed is a measure of LH rather than of FSH, but other evidence is against this supposition (*see* previous discussion). Alternatively, in addition to LH, FSH might be a necessary factor for the formation of Leydig cells in man.

Significance of thickening of the tunica propria of the tubules

It has been pointed out that thickening of the tunica propria of the tubules occurs in those eunuchoid patients who in the past have had a

regression in pituitary stimulation. Thus, it is present in those patients with destructive lesions of the pituitary, in whom the process started after puberty. When present in hypogonadal patients without a demonstrable lesion of the pituitary, there is often a history suggesting a greater degree of pituitary stimulation at some time in the past. Whereas one usually considers a lesion of this nature irreversible, in Case 12 (*see* Appendix) the tunica propria thickening almost disappeared after testosterone therapy.

Lipoid accumulation in the Sertoli cells

The cause of this accumulation remains uncertain, but it is apparently correlated with an arrest of FSH secretion after full development of the testicular tubules. It may represent an intracellular accumulation of "X" hormone which is not released due to the absence of FSH (*cf.* accumulation of cholesterol in the adrenal cortex of hypophysectomized rats (96)). Similar deposits of lipoid, however, are not reported by Huggins and Russell (97) in the testes of mature dogs after hypophysectomy.

SUMMARY AND CONCLUSIONS

1. In this study of testicular deficiency in 141 men, histologic examinations of testes have been correlated with clinical findings and excretions of urinary follicle-stimulating hormone (FSH) and 17-ketosteroids.

2. The cases have been classified into 3 groups: those with (A) low, (B) normal and (C) high FSH excretion, respectively.

3. Group A was subdivided according to whether the low FSH excretion was idiopathic, secondary to organic lesions in or near the pituitary, or secondary to estrogen therapy. If pituitary stimulation had never occurred, the testes resembled prepubertal glands. If testicular maturity was attained prior to the decrease in pituitary stimulation, the histologic features were hypospermatogenesis, lipoid accumulation in the Sertoli cells, thickening of the tubular tunica propria, deposition of peritubular collagen and elastic tissue and absence of normal Leydig cells.

4. Group B was divided into two subgroups according to whether there was an associated endocrine disturbance or not. The first subgroup comprised cases in which the endocrine disturbance was thought to be due to lack of luteinizing hormone, and cases with acromegaly; the second subgroup comprised cases with spermatogenic failure only, *viz.*, arrest of maturation, hypospermatogenesis, and cases with oligospermia but with normal testicular histologic structure.

5. Group C contained cases with absence of germ cells but otherwise normal testicular microscopic anatomy, sclerosing tubular degeneration, absence of testes, the male "climacteric," mumps orchitis, and a few miscellaneous syndromes.

6. These studies suggest that there are two testicular hormones, one made by the Leydig cells and a second by the tubules; that it is the Sertoli cells and not the germ cells which make this second hormone; and that the functions of the second or "X" hormone are to support spermatogenesis, to inhibit FSH production, to stimulate other gonadotropins (? luteinizing hormone), and possibly to exert an inhibitory effect on mammary growth.

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REFERENCES

1. Hotchkiss, R. S.: Fertility in Men. Philadelphia, J. B. Lippincott Company, 1944.
2. Charny, C. W.: Testicular biopsy; its value in male sterility, *J.A.M.A.* **115:** 1429–1432 (Oct. 26) 1940.
3. Hotchkiss, R. S.: Testicular biopsy in the diagnosis and treatment of sterility in the male, *Bull. New York Acad. Med.* **18:** 600–605 (Sept.) 1942.
4. Charny, C. W., and Meranze, D. R.: Testicular biopsy; further studies in male infertility, *Surg., Gynec. & Obst.* **74:** 836–842 (Apr.) 1942.
5. Charny, C. W.: The testicular biopsy. A five-year survey, *in:* Conference on Diagnosis in Sterility, edited by E. T. Engle, Springfield, Illinois, Charles C Thomas, Publisher, 1946, p. 43–66.
6. Engle, E. T.: The testis biopsy in infertility, *J. Urol.* **57:** 789–798 (May) 1947.
7. Albright, F.; Forbes, A. P.; Fraser, R.; Miller, R. B., and Reifenstein, E. C., Jr.: A classification of the causes of hypoleydigism, *Tr. A. Am. Physicians* **56:** 43–54, 1941.
8. Heller, C. G.: Prepuberal hypogonadotropic eunuchoidism; criteria for diagnosis, and discussion of pathological physiology and treatment, *Proc. Am. Federation Clin. Research* **2:** 97, 1945.
9. Heller, C. G.; Nelson, W. O., and Roth, A. A.: Functional prepuberal castration in males, *J. Clin. Endocrinol.* **3:** 573–588 (Nov.) 1943.
10. Klinefelter, H. F. Jr.; Reifenstein, E. C., Jr., and Albright, F.: Syndrome characterized by gynecomastia, aspermatogenesis without a-leydigism and increased excretion of follicle-stimulating hormone, *J. Clin. Endocrinol.* **2:** 615–627 (Nov.) 1942.
11. Heller, C. G., and Nelson, W. O.: Hyalinization of the seminiferous tubules associated with normal or failing Leydig-cell function. Discussion of relationship to eunuchoidism, gynecomastia, elevated gonadotrophins, depressed 17-ketosteroids and estrogens, *J. Clin. Endocrinol.* **5:** 1–12 (Jan.) 1945.
12. Nelson, W. O., and Heller, C. G.: Hyalinization of the seminiferous tubules associated with normal or failing Leydig-cell function. Microscopic picture in the testis and associated changes in the breast, *J. Clin. Endocrinol.* **5:** 13–26 (Jan.) 1945.

13. WERNER, A. A.: The male climacteric, *J.A.M.A.* **112:** 1441–1443 (Apr. 15) 1939.
14. HELLER, C. G., and MYERS, G. B.: The male climacteric. Its symptomatology and treatment, *J.A.M.A.* **126:** 472–477 (Oct. 21) 1944.
15. MCCULLAGH, E. P.: Climacteric—male and female, *Cleveland Clin. Quart.* **13:** 166–176 (July) 1946.
16. NELSON, W. O.: Studies on the testes of hypogonadal men, *J. Omaha Mid-West Clin. Soc.* **8:** 1–7 (Jan.) 1947.
17. NELSON, W. O.: The physiological basis of hypogonadism in the male, *M. Clin. North America* **32:** 97–110 (Jan.) 1948.
18. WERNER, S. C.: Clinical syndromes associated with gonadal failure in men, *Am. J. Med.* **3:** 52–66 (July) 1947.
19. MCCULLAGH, E. P.: Sex hormone deficiencies—some clinical considerations, *in:* Recent Progress in Hormone Research, New York, Academic Press, 1948, Vol. 2, pp. 295–344.
20. HELLER, C. G., and MADDOCK, W. O.: The clinical uses of testosterone in the male, *in:* Vitamins and Hormones, New York, Academic Press, 1947, Vol. 5, pp. 393–432.
21. HELLER, C. G., and NELSON, W. O.: Classification of male hypogonadism and a discussion of the pathologic physiology, diagnosis and treatment, *J. Clin. Endocrinol.* **8:** 345–366 (May) 1948.
22. MCCULLAGH, E. P.: Testicular dysfunction, *Bull. New York Acad. Med.* **24:** 341–363 (June) 1948.
23. SIMMONS, F. A., and SNIFFEN, R.: Testicular biopsy in the sterile male, *West. J. Surg.* **55:** 508–517 (Sept.) 1947.
24. HUHNER, M.: Aspiration of the testicles in the diagnosis and prognosis of sterility, *J. Urol.* **19:** 31–41 (Jan.) 1928.
25. MICHELSON, L.: Treatment of azoospermia by vaso-epididymal anastomosis, *West. J. Surg.* **55:** 120–128 (Feb.) 1947.
26. WILLIAMS, W. W., and SIMMONS, F. A.: The clinical approach to the diagnosis of sterility, *Urol. & Cutan. Rev.* **46:** 558–570 (Sept.) 1942.
27. SIMMONS, F. A.: Clinical interpretation of the semen analysis, *in:* Conference on Diagnosis in Sterility, edited by E. T. Engle, Springfield, Illinois, Charles C Thomas, Publisher, 1946, pp. 16–42.
28. FRASER, R. W.; FORBES, A. P.; ALBRIGHT, F.; SULKOWITCH, H., and REIFENSTEIN, E. C. JR.: Colorimetric assay of 17-ketosteroids in urine, *J. Clin. Endocrinol.* **1:** 234–256 (March) 1941.
29. KLINEFELTER, H. F., JR.; ALBRIGHT, F., and GRISWOLD, G. C.: Experience with a quantitative test for normal or decreased amounts of follicle stimulating hormone in the urine in endocrinological diagnosis, *J. Clin. Endocrinol.* **3:** 529–544 (Oct.) 1943.
30. MCMANUS, J. F. A.: The periodic acid routine as applied to the kidney, *Am. J. Path.* **24:** 643–648 (May) 1948.
31. BLACKMAN, S. S., JR.: Concerning the function and origin of the reticular zone of the adrenal cortex: hyperplasia in the adrenogenital syndrome, *Bull. Johns Hopkins Hosp.* **78:** 180–218 (Apr.) 1946.
32. SIMPSON, M. E., and EVANS, H. M.: Comparison of the spermatogenic and androgenic properties of testosterone propionate with those of pituitary ICSH in hypophysectomized 40-day-old male rats, *Endocrinology* **39:** 281–285 (Nov.) 1946.
33. MASON, H. L.; POWER, M. H.; RYNEARSON, E. H.; CIARAMELLI, L. C.; LI, C. H., and EVANS, H. M.: Results of administration of anterior pituitary adrenocorticotropic hormone to a normal human subject, *J. Clin. Endocrinol.* **8:** 1–14 (Jan.) 1948.

34. FORSHAM, P. H.; THORN, G. W.; PRUNTY, F. T. G., and HILLS, A. H.: Clinical studies with pituitary adrenocorticotropin, *J. Clin. Endocrinol.* **8**: 15–66 (Jan.) 1948.
35. BARTTER, F. C.: Does one pituitary hormone stimulate all three functions of the adrenal cortex? *J. Clin. Investigation* **27**: 523 (July) 1948.
36. MCALPINE, H. T.; VENNING, E. H.; JOHNSON, L.; SCHENKER, V.; HOFFMAN, M. M., and BROWNE, J. S. L.: Metabolic changes following the administration of pituitary adrenocorticotropic hormone (ACTH) to normal humans, (abstract) *J. Clin. Endocrinol.* **8**: 591 (July) 1948.
37. ALBRIGHT, F.; SMITH, P. H., and FRASER, R.: A syndrome characterized by primary ovarian insufficiency and decreased stature, *Am. J. M. Sc.* **204**: 625–648 (Nov.) 1942.
38. ALBRIGHT, F.: The effect of hormones on osteogenesis in man, *in:* Recent Progress in Hormone Research, New York, Academic Press, 1947, Vol. 1, pp. 293–353.
39. TALBOT, N. B.; BUTLER, A. M.; BERMAN, R. A.; RODRIGUEZ, P. M., and MACLACHLAN, E. A.: Excretion of 17-ketosteroids by normal and by abnormal children, *Am. J. Dis. Child.* **65**: 364–375 (Mar.) 1943.
40. MOORE, C. R., and PRICE, D.: Gonad hormone function and the reciprocal influence between gonad and hypophysis with its bearing on the problem of sex-hormone antagonism, *Am. J. Anat.* **50**: 13–71 (March) 1932.
41. DUNN, C. W.: Stilbestrol induced testicular degeneration in hypersexual males, *J. Clin. Endocrinol.* **1**: 643–648 (Aug.) 1941.
42. SEGALOFF, A.: Testicular dysfunction: diagnosis and treatment, *Am. Pract.* **1**: 15–22 (Sept.) 1946.
43. MILLS, R. G.: The pathological changes in the testes in epidemic pneumonia, *J. Exper. Med.* **30**: 505–529 (Nov.) 1919.
44. MCLEOD, J., and HOTCHKISS, R. S.: The effect of hyperpyrexia upon spermatozoa counts in man, *Endocrinology* **28**: 780–784 (May) 1941.
45. DEL CASTILLO, E. B.; TRABUCCO, A., and DE LA BALZE, F. A.: Syndrome produced by absence of the germinal epithelium without impairment of the Sertoli cells, *J. Clin. Endocrinol.* **7**: 493–502 (July) 1947.
46. LEROY, G. V.: The medical sequelae of the atomic bomb explosion, *J.A.M.A.* **134**: 1143–1148 (Aug. 2) 1947.
47. TRABUCCO, A.: Esterilidad congenita en el hombre (Congenital sterility in man), *Medicina, Buenos Aires* **5**: 369–387 (July) 1945.
48. HELLER, C. G., and NELSON, W. O.: Hyalinization of seminiferous tubules and clumping of Leydig cells. Notes on treatment of the clinical syndrome with testosterone propionate, methyl testosterone and testosterone pellets, *J. Clin. Endocrinol.* **5**: 27–33 (Jan.) 1945.
49. REIFENSTEIN, E. C. JR.: Hereditary familial hypogonadism, *Proc. Am. Federation Clin. Research* **3**: 86, 1947.
50. SNIFFEN, R. C.; HOWARD, R. P., and SIMMONS, F. A.: To be published.
51. HUGGINS, C., and CLARK, P. J.: Quantitative studies of prostatic secretion. II. The effect of castration and of estrogen injection on the normal and on the hyperplastic prostate gland of dogs, *J. Exper. Med.* **72**: 747–762 (Dec.) 1940.
52. MCCULLAGH, D. R., and WALSH, E. L.: Experimental hypertrophy and atrophy of the prostate gland, *Endocrinology* **19**: 466–470 (July–Aug.) 1935.
53. LEREBOULLET, L.: Contribution à l'étude des atrophies testiculaires et des hypertrophies mammaires observées à la suite de certaines orchites (féminisme) (Contribution to the study of testicular atrophies and of mammary hypertrophies observed following certain cases of orchitis (feminism), *Gaz. hebd. de méd. et de chir.* **14**: 533–535 (Aug. 24) and 549–551 (Aug. 31) 1877.

54. LAIGNEL-LAVASTINE, and COURBON, P.: Féminisme post-ourlien (microrchidie et gynécomastie consécutives à une orchite double) (Feminism following mumps (small testes and gynecomastia after bilateral orchitis)), *Bull. et mém. Soc. méd. d'hôp. de Paris* **41**: 914–915 (July 20) 1917.
55. BÉNARD, R.: La stérilité consécutive aux oreillons de l'adulte est un mythe (Sterility following mumps in the adult is a myth), *Médecine* **9**: 184–189 (Dec.) 1927.
56. WAGENSEIL, F.: Beiträge zur Kenntnis der Kastrationsfolgen und des Eunuchoidismus beim Mann (Contributions to knowledge of the results of castration and of eunuchoidism in man), *Ztschr. f. Morphol. u. Anthropol.* **26**: 264–304, 1927.
57. WAGENSEIL, F.: Chinesische Eunuchen (Chinese eunuchs), *Ztschr. f. Morphol. u. Anthropol.* **32**: 415–468, 1933.
58. WOOLLEY, G. W.: Gonadectomy and adrenal cortical tumors, *Tr. Josiah Macy, Jr. Foundation Conferences*, Conference on Metabolic Aspects of Convalescence, 11th Meeting, Oct. 1945, pp. 77–86.
59. WOOLLEY, G. W.: Control of nodular hyperplasia and adrenal cortical carcinoma by steroid compounds, *Tr. Josiah Macy, Jr. Foundation Conferences*, Conference on Metabolic Aspects of Convalescence, 13th Meeting, June 1946, pp. 111–115.
60. KLINEFELTER, H. F. JR.: Personal communication.
61. BENDA, C. E., and BIXBY, E. M.: Urinary excretion of 17-ketosteroids in various conditions of oligophrenia correlated with some autopsy observations, *J. Clin. Endocrinol.* **7**: 503–518 (July) 1947.
62. HELLER, C. G.: Discussion on paper given by the authors, Association for the Study of Internal Secretions, 30th meeting, Chicago, June, 1948.
63. GREEP, R. O.; VAN DYKE, H. B., and CHOW, B. F.: Gonadotropins of the swine pituitary. 1. Various biological effects of purified thylakentrin (FSH) and pure metakentrin (ICSH), *Endocrinology* **30**: 635–649 (May) 1942.
64. SIMPSON, M. E.: Gonadotrophic hormones with special reference to their action on the female reproductive mechanism, *West. J. Surg.* **52**: 287–294 (July) 1944.
65. HELLER, C. G., and HELLER, E. J.: Gonadotropic hormone: clinical application of extraction methods for assay purposes, *Endocrinology* **24**: 319–325 (March) 1939.
66. JUNGCK, E. C.; MADDOCK, W. O., and HELLER, C. G.: Gonadotropic hormone: comparison of ultrafiltration and alcohol-precipitation methods of recovery from urine, *J. Clin. Endocrinol.* **7**: 1–10 (Jan.) 1947.
67. FEVOLD, H. L., and HISAW, F. L.: Interactions of gonad stimulating hormone in ovarian development, *Am. J. Physiol.* **109**: 655–665 (Oct.) 1934.
68. HAMBURGER, C.: Studies on gonadotrophic hormones from the hypophysis and chorionic tissue, *Acta path. et microbiol. Scandinav. Suppl.* **17**: 1–184, 1933.
69. EVANS, H. M.; SIMPSON, M. E.; TOLKSDORF, S. and JENSEN, H.: Biological studies of the gonadotropic principles in sheep pituitary substance, *Endocrinology* **25**: 529–546 (Oct.) 1939.
70. SIMPSON, M. E.: Unpublished data
71. KATZMAN, P. A., and DOISY, E. A.: The quantitative determination of small amounts of gonadotrophic material. *J. Biol. Chem.* **106**: 125–139 (Aug.) 1934.
72. CATCHPOLE, H. R.; GREULICH, W. W., and SOLLENBERGER, R. T.: Urinary excretion of follicle-stimulating hormone in young and adolescent boys, *Am. J. Physiol.* **123**: 32–33 (July) 1938.
73. GOLDZIEHER, J. W., and HAMBLEN, E. C.: Andrologic endocrinology. Correlations of clinical symptoms, urinary hormone excretion and testicular cytology, *Surg. Gynec. & Obst.* **85**: 583–596 (Nov.) 1947.

74. LLOYD, C. W.; MORLEY, M.; MORROW, K.; LOBOTSKY, J., and HUGHES, E. C.: Estimation of urinary gonadotropin of the nonpregnant human by the mouse uterine weight and ovarian hyperemia responses, *J. Clin. Endocrinol.* **9:** 636–645 (July) 1949.
75. HELLER, C. G.: HELLER, E. J., and SEVRINGHAUS, E. L.: Does estrogen substitution materially inhibit pituitary gonadotropic potency? *Endocrinology* **30:** 309–316 (Feb.) 1942.
76. HELLER, C. G.: MADDOCK, W. O.; JUNGCK, E. C., and NELSON, W. O.: The role of the Sertoli cell in the testis-pituitary axis in men, *Federation Proc.* **8:** 71–72, 1949.
77. MCCULLAGH, D. R.: Dual endocrine activity of the testes, *Science* **76:** 19–20 (July 1) 1932.
78. CATCHPOLE, H. R.; HAMILTON, J. B., and HUBERT, G. R.: Effect of male hormone therapy on urinary gonadotropins in man, *J. Clin. Endocrinol.* **2:** 181–186 (March) 1942.
79. REIFENSTEIN, E. C., JR.: Discussion *in:* Recent Progress in Hormone Research, New York, Academic Press, 1948, vol. 2, p. 335.
80. MCCULLAGH, E. P., and HRUBY, F. J.: Testis-pituitary interrelationship. The relative inability of testosterone to reduce urinary gonadotropin in eunuchoid men, *J. Clin. Endocrinol.* **9:** 113–125 (Feb.) 1949.
81. MOTTRAM, J. C., and CRAMER, W.: On the general effects of exposure to radium on metabolism and tumor growth in the rat and special effects on testes and pituitary, *Quart. J. Exper. Physiol.* **13:** 209–226, 1923.
82. MARTINS, T., and ROCHA, A.: The regulation of the hypophysis by the testicle and some problems of sexual dynamics (experiments with parabiotic rats), *Endocrinology* **15:** 421–434 (Sept.–Oct.) 1931.
83. TÖRNBLOM, N.: Internal Secretion of the Germinal Tissue of the Testes and Prostatic Hypertrophy. Uppsala, Almqvist and Wiksells, 1942.
84. MCCULLAGH, E. P.; SCHNEIDER, R. W.; BOWMAN, W., and SMITH, M. B.: Adrenal and testicular deficiency. A comparison based on similarities in androgen deficiency, androgen and 17-ketosteroid excretion, and on differences in their effects upon pituitary activity, *J. Clin. Endocrinol.* **8:** 275–294 (Apr.) 1948.
85. HUGGINS, C., and MOULDER, P. V.: Estrogen production by Sertoli cell tumors of the testis, *Cancer Research* **5:** 510–514 (Sept.) 1945.
86. RUZICKA, L., and PRELOG, V.: Untersuchungen von Extracten aus Testes. Zur Kenntnis der Lipoide aus Schweinetestes (Analyses of extracts from testes. Information on the lipoids from hog testes), *Helvet. chim. acta* **26:** 975–994, 1943.
87. SELYE, H.: The pharmacology of steroid hormones and their derivatives, *Rev. canad. de biol.* **1:** 577–632 (Aug.) 1942.
88. SELYE, H.: Textbook of Endocrinology. Montreal, Acta Endocrinologica, Inc., 1947, p. 633.
89. ALBERT, S., and SELYE, H.: The effect of various pharmacological agents on the morphogenetic actions of estradiol, *J. Pharmacol. & Exper. Therap.* **75:** 308–315 (Aug.) 1942.
90. SCAGLIONE, S.: Ormone follicolare e ovaio (Follicular hormone and ovary), *Riv. ital. di ginec.* **11:** 463–471 (Nov.) 1930.
91. FEVOLD, H. L.: *in:* Sex and Internal Secretions, edited by E. Allen, Baltimore, Williams and Wilkins, ed. 2., 1939, Chap. 17.
92. ASTWOOD, E. B.: The regulation of corpus luteum function by hypophysial luteotrophin, *Endocrinology* **28:** 309–320 (Feb.) 1941.

93. HELLBAUM, A. A., and GREEP, R. O.: Action of estrogen on release of hypophyseal leuteinizing hormone, *Proc. Soc. Exper. Biol. & Med.* **63**: 53–56 (Oct.) 1946.
94. HISAW, F. L.: Development of the Graafian follicle and ovulation, *Physiol. Rev.* **27**: 95–119 (Jan.) 1947.
95. LIPSCHUTZ, A.; IGLESIAS, R.; BRUZZONE, S.; HUMEREZ, J., and PEÑARANDA, J. M. Progesterone and desoxycorticosterone in the steroid control of the gonadotrophic function of the hypophysis, *Endocrinology* **42**: 201–209 (March) 1948.
96. SAYERS, G.; SAYERS, M. A.; WHITE, A., and LONG, C. N. H.: Effect of pituitary adrenocorticotrophic hormone on cholesterol content of rat adrenal glands, *Proc. Soc. Exper. Biol. & Med.* **52**: 200–202 (March) 1943.
97. HUGGINS, C., and RUSSELL, P. S.: Quantitative effects of hypophysectomy on testis and prostate of dogs, *Endocrinology* **39**: 1–7 (July) 1946.
98. LERMAN, J., and MEANS, J. H.: Hypopituitarism associated with epilepsy following head injury, *J. Clin. Endocrinol.* **5**: 119–131 (March) 1945.

APPENDIX

Clinical summaries

Group A. Conditions with low FSH Excretion

i. *Idiopathic eunuchoidism-with-low-FSH*

Case 1 (U-407367). This Italian[4] student, who was 16 years old in 1945, showed pubertal changes except for scant pubic hair which had appeared two years earlier. He had erections but no ejaculations. *Examination.* No beard or axillary hair and but scant pubic hair. Phallus and testes were infantile, and his voice was high pitched. By 1947 his height was 69½ in., span 74¼ in. and weight 173 lbs. His genitalia were unchanged and his prostate was not palpable. The breasts were flat.[5] *Treatment.* None prior to 1947. *Laboratory findings.* X-ray examination showed a normal sella. When his chronologic age was 17 years, his bone age was 13 years. Semen was not obtained. Urinary 17-ketosteroids (5 tests) averaged 2.9 mg. per day. FSH assays, by dialysis method on three occasions between 1945 and 1946, were consistently less than 6.5 m.u. per day. *Testicular biopsy* (45-5096). The right testis in July 1945 resembled a prepubertal gonad (similar to that shown in Figs. 2 and 4).

Case 7 (U-8440). The patient was a Polish laborer, who was 21 years old in 1941. In 1937, when aged 17, his height was 61½ in., span 63½ in. and weight 99 lbs. He had no beard, axillary or pubic hair; the phallus was 2.5 cm. long; the prostate and the right testis were not palpable and the left testis was bean-sized. The breasts were flat, his voice was high pitched and he had no erections. *Examination.* In 1941 his height was 65 in. and weight 124 lbs. He showed adult secondary sex characters but the testes remained unchanged. *Treatment.* He received thyroid therapy in 1935; courses of chorionic gonadotropin in 1936, 1937 and 1939; and courses of testosterone propionate by injections in 1938 and 1939, and later by pellets, the last implantation before biopsy being 300 mg. in December 1940. *Laboratory findings.* X-ray examination showed a normal sella in 1935 and in 1948. The B.M.R. was −20 per cent in 1935. Semen not obtained. Urinary 17-ketosteroids (9 tests) averaged 2.8 mg. per day in 1939–40 in intervals when androgen therapy was omitted. In 1935, the Prolan A test gave negative results. FSH assay

[4] Racial origins, not nationalities, are given in this and the following case summaries.

[5] "Breasts flat" or "no gynecomastia" is to be regarded as a clinical impression. Breasts biopsies were made only in those cases where they are specifically mentioned.

by the non-dialysis method in September 1939 was less than 5 m.u. per 100 cc. and in December 1941 and January 1942, less than 10 m.u. per 100 cc.; by the dialysis method in June 1942 it was less than 6.5 m.u. per day. *Testicular biopsy* (41-7751). The left testis in December 1941 showed an immature structure (Fig. 4). This case was reported as Case 74 by Fraser and associates (28).

Case 8 (U-218519). (For details *see* Case 47 of Fraser and co-workers (28).) This Italian patient, who was 40 years old in 1941, prior to treatment in 1940 had no erections and showed no sexual development. Laboratory findings and testicular biopsy results were comparable to those in Case 7.

Case 12 (U-545631). This English student was 19 years old in 1946. Axillary and pubic hair appeared at 14 years. He had rare erections without emissions. *Examination.* His height was 65 in., span 67¼ in. and weight 137 lbs. He had no beard and there was no temporal hair recession. A moderate amount of axillary and pubic hair was present but there was very scant regrowth in the axillae after shaving. His voice was high pitched. Phallus, testes and prostate were very small. There was no gynecomastia. *Treatment.* None before 1946. *Laboratory findings.* X-ray examination showed a normal sella, and delayed epiphyseal union at the wrists. The insulin tolerance test gave normal results. Semen was not obtained. Urinary 17-ketosteroids (2 tests) averaged 5.9 mg. per day. FSH assays by the dialysis method on two occasions in 1946 were more than 6.5 m.u. and less than 13 m.u. per day. *Testicular biopsy* (46-6772). The right testis in September 1946 showed marked thickening of the tunica propria, though there was only slight maturation of the seminiferous epithelium, and there were no Leydig cells. *Follow-up note.* Between December 1946 and November 1947 inclusive, he received a total of 1050 mg. of testosterone by subcutaneous implantation. *Testicular biopsy* (48-1143) in February 1948 showed decreased thickening of the tunica propria and increased maturation of the germinal epithelium up to the stage of primary spermatocytes. There were no Leydig cells.

Case 15 (U-22376). The patient, a Hebrew butcher, was 47 years old in 1943. He had been married eighteen years without children, and had been impotent since 1932, before which he had some erections with scanty ejaculations. Pubic hair appeared at 14 years. In 1935 his height was 66¾ in., span 75¾ in. and weight 181 lbs. He had no beard or axillary hair and only scant pubic hair. Phallus and testes were small and the prostate was not palpable. The breasts were flat. His voice was somewhat high pitched. *Examination.* In 1943 his condition was unchanged, except for some phallic enlargement and increased secondary sex hair. *Treatment.* He received a course of chorionic gonadotropin in 1935, and also thyroid therapy. In 1938, he received testosterone propionate therapy for ten months, but no further treatment for five years prior to biopsy. *Laboratory findings.* A roentgenogram showed the sella to be normal. The B.M.R. was −21 per cent in 1935. A few sperm were seen by the referring physician in 1934, but none was present in 1938. Urinary 17-ketosteroids in 1943 were 9.1 mg. per day. The Prolan A test gave negative results in 1935. FSH assay by the dialysis method in 1943 was less than 6.5 m.u. per day. While receiving testosterone therapy in 1945 and 1947, FSH assays were more than 6.5 m.u. but less than 13 m.u. per day. *Testicular biopsy* (43-5880). The testis in September 1943 showed the adult type of tubules with mature Sertoli cells, though poor germinal activity and very rare sperm. There was slight thickening of the tunica propria and peritubular proliferation. Only rare Leydig cells were recognizable, and these showed a remarkable foamy cytoplasm with an unusual number of crystalloids.

Case 18 (U-526451). A French-Canadian machinist, 35 years old in 1946, had been married the second time five years previously and was without children by either mar-

riage. He had normal potency but a very scant ejaculate. Bilateral breast enlargements were removed in 1944 at a local hospital; the diagnosis was cystic mastitis. *Examination.* His height was 61 in., span 64 in. and weight 182 lbs. The beard was scant, but pubic and axillary hair appeared normal, although showing poor regrowth after shaving. Phallus and testes were slightly smaller than those of the average adult and the prostate was very small. His voice was high pitched. *Treatment* consisted of a short course of testosterone therapy in April 1945; there was no more treatment for the next eighteen months. *Laboratory findings.* A roentgenogram showed a normal sella. The B.M.R. was −17 per cent An insulin tolerance test gave normal results. The referring physician found no sperm in 1945. Urinary 17-ketosteroids (3 tests) averaged 4.7 mg. per day. FSH assay in May 1946 by the non-dialysis method was less than 96 m.u. per day, and on two subsequent occasions after omission of treatment in 1946, by the dialysis method, assays were less than 6.5 m.u. per day. *Testicular biopsy* (46-3440). The right testis in May 1946 showed adult tubules with mature Sertoli cells, very poor germinal activity, slight thickening of tunica propria and peritubular proliferation, and no Leydig cells.

ii. *Low FSH secondary to organic lesions in or near the pituitary*

Case 20 (U-13519). This patient was an unemployed Englishman, who was 19 years old in 1947. A suprasellar tumor was first diagnosed ten years earlier. Nearly blind, he had not matured sexually except for the development of some sexual hair. *Examination.* His height was 58 in., span 61 in. and weight 118 lbs. He had no beard and there was no temporal hair recession: there was moderate axillary and scant pubic hair. His phallus was infantile and the testes only 2 cm. long. He had a very small prostate, flat breasts and a high pitched voice. *Treatment.* No endocrine therapy. *Laboratory findings.* Insulin tolerance was normal. Semen was not obtained. Urinary 17-ketosteroids in February 1947 were 11.2 mg. per day, but in July 1947 were 2.1 mg., which is probably more accurate. FSH assay by the dialysis method in February 1947 was less than 6.5 m.u. and in April 1947 was less than 3 m.u. per day. *Testicular biopsy* (47-3447). The testis in April 1947 showed undifferentiated infantile tubules, slight thickening of tunica propria and no Leydig cells. *Exploration.* Craniotomy in April 1947 by Dr. C. White revealed a large suprasellar craniopharyngioma.

Case 21 (U-579404). A single, English, machine worker was 28 years old in 1947. Pubic hair appeared at 15 years, but erections and voice changes occurred only after treatment with tablets (androgen?) in 1944. Recently frequent headaches had occurred. *Examination.* His height was 64 in., span 68 in. and weight 138 lbs. He had no beard, no temporal hair recession, no axillary hair, and but scant pubic hair. Phallus and testes were small and the prostate barely palpable. The breasts were flat and his voice had become deep. *Laboratory findings.* A roentgenogram of the skull showed a sellar cyst extending into the sphenoid sinus. Epiphyseal closure was late. Insulin tolerance was normal. Semen was not obtained. Urinary 17-ketosteroids were 2.4 mg. per day. FSH assay in June 1947 by the dialysis method was less than 6.5 m.u. per day. *Testicular biopsy* (47-5128). The right testis in June 1947 showed cord-like tubules of partially differentiated Sertoli cells and inactive germ cells, pronounced thickening of the tunica propria, and no Leydig cells (Fig. 5). *Exploration.* By the endonasal approach, the cyst was drained in September 1947 by Dr. O. Hirsch.

Case 24 (U-162810). A French-Canadian tailor with 8 children was 75 years old in 1946. In 1926 he noted weakness and decreased body hair; in 1930, cold intolerance; and in 1934, impotence and attacks of vomiting. There were 7 hospital admissions from 1934 to 1946 for myxedema and finally for cardiac failure. *Examination.* His height was 64½ in.

and weight 137 lbs. He shaved twice weekly, had no axillary hair and but scant pubic hair. Phallus and testes were small and the prostate was not palpable. His breasts were flat. Visual fields were normal in 1940. *Treatment.* Thyroid therapy during the period 1940–46. He received testosterone propionate intermittently, beginning in May 1940 and stopping in May 1943, and also adrenal cortical extract 1945–46. *Laboratory findings.* X-ray examination showed a normal sella. In 1943 he had a low serum sodium, positive Wilder test and hypoglycemia unresponsiveness. Urinary 17-ketosteroids in 1940 were 3.0 mg. and in 1943, 0.9 mg. per day. FSH assay by the non-dialysis method in April 1940 was less than 10 m.u. per 100 cc., and by the dialysis method in August 1943, less than 3 m.u. per day. *Postmortem examination* (A-11926), in 1946 showed an intrasellar pituitary chromophobe adenoma and atrophy of the thyroid, adrenals, prostate and testes. The testes showed marked interstitial fibrosis and tubular sclerosis, so that only rare tubules containing a few fat-laden Sertoli cells remained. No Leydig cells were seen.

Case 26 (U-429846). An English carpenter with 1 child was 64 years old in 1944. In 1920 a right orchidectomy was done for hydrocele, and the left side aspirated. In 1942 he suffered an ocular palsy, became cold-sensitive and lost libido. *Examination.* His height was 66 in. and weight 127 lbs. His beard was normal, but axillary hair was absent and pubic hair was scant. Phallus, left testis, prostate, breasts and voice were normal. He had a bitemporal hemianopsia. *Treatment.* No endocrine therapy before study. *Laboratory findings.* A roentgenogram showed decalcification of the clinoids and of the sellar floor, without enlargement. The B.M.R. was −20 per cent. Semen was not studied. Urinary 17-ketosteroids were 0.6 mg. per day. FSH assay in January 1944 by the dialysis method was less than 3 m.u. per day. *Testicular biopsy* (44-1102). The left testis in February 1944 showed few spermatogonia but many fat-laden Sertoli cells, thickened tunica propria, peritubular proliferation and no Leydig cells (Fig. 6). *Exploration.* No craniotomy. Died at home in June 1944.

Case 28 (U-122676). (Reported as Case 57 by Fraser and co-workers (28).) *Testes* (A-9238) resembled those in Figure 6.

Case 30 (U-85832). (Presented in detail as second patient by Lerman and Means (98).) This man, who was 50 years old in 1944, had hypopituitarism following a head injury. *Testicular biopsies* (44-98 and 44-1222) and postmortem specimens (A-11210) showed essentially the same findings as those shown in Figure 6.

Case 31 (U-68854). (Reported as Case 22 by Klinefelter, Albright and Griswold (29).) After testosterone pellet implantation in July 1947, FSH assay by the dialysis method was greater than 3 m.u. but less than 6.5 m.u. per day. *Testicular biopsy* (42-1914). Findings similar to those in Case 32.

Case 32 (U-550872). This Polish patient, who was 41 years old in 1947, had been married for 19 years; his wife miscarried once in 1934. He was normally developed but had had failing vision and decreased potency for four years. *Examination.* His height was 67 in., span 69½ in., and weight 165 lbs. His beard was normal but the axillary hair regrowth rate was poor and pubic hair was sparse. Phallus, testes, breasts and voice were normal. The prostate was not palpable. Visual fields were constricted. *Treatment.* No endocrine therapy prior to study. *Laboratory findings.* X-ray examination showed erosion and widening of the sella. The epiphyses of the hand were closed. The B.M.R. was −25 per cent. An insulin tolerance test showed hypoglycemia unresponsiveness. Semen was not studied. Urinary 17-ketosteroids (4 tests) averaged 1.1 mg. per day. FSH assay by the dialysis method in October 1946 was less than 6.5 m.u., and in March 1947 was more than 3 m.u. per day. *Testicular biopsy* (47-147). The left testis in January 1947 showed tubules varying from nearly normal to relatively inactive. There was slight lipoid accumulation in the

Sertoli cells, slight thickening of the tunica propria, slight peritubular proliferation and decreased numbers of Leydig cells. *Exploration.* In March 1947 Dr. O. Hirsch, using the intranasal route, found a solid chromophobe adenoma.

iii. *Low FSH secondary to estrogen therapy*

Case 33 (U-380940). (*See* Case 43 following, for early history.) *Research treatment.* Estradiol dipropionate, 5 mg. every third day, was started on April 27, 1943, and increased to 10 mg. daily on May 21, the last dose being on June 7, 1943. The total dose was 220 mg. in forty-two days. During therapy, urinary 17-ketosteroids were unaltered, 7 tests averaging 9.5 mg. per day; but FSH assay by the dialysis method on June 15 was less than 3 m.u. per day. *Testicular biopsy* (43-3445). The left testis on June 8, 1943, showed a marked reduction in spermatogenesis, some increased lipoid in the Sertoli cells, slight thickening of the tunica propria, and no typical Leydig cells. This picture resembled, but showed less marked changes than that illustrated in Figure 7.

Case 34 (U-334479). This married English retired executive, with no children, was 78 years old in 1942. He had carcinoma of the prostate with diffuse osseous metastases. *Examination.* His weight was 165 lbs. Hair, breasts and phallus were normal. The testes were slightly small and soft. *Treatment.* No endocrine therapy before first biopsy. *Laboratory findings.* The semen was not studied. Urinary 17-ketosteroids (6 tests) averaged 4.2 mg. per day. FSH assay by the non-dialysis method was greater than 10 m.u. per 100 cc. *Testicular biopsy* (42-1171). The right testis in February 1942 showed fully active spermatogenesis with scattered islands of normal Leydig cells. *Research treatment.* Beginning March 12, 1942, he was given estradiol benzoate 1.66 mg. daily, increased on April 7 to 3.3 mg. daily. The total dose in sixty-seven days was 180 mg. During therapy, 17-ketosteroids were unchanged at an average of 4.0 mg. per day, but FSH assay by the dialysis method on March 31 was less than 13 m.u. and on April 27, less than 6.5 m.u. per day. *Testicular biopsy* (42-3260). Both testes on May 18, 1942, showed tubules with inactive spermatogonia but considerable lipoid accumulation in the Sertoli cells. There were also increased thickening of the tunica propria, peritubular fibrosis and a marked decrease in Leydig cells (Fig. 7). *Subsequent course.* Therapy was changed to large doses of diethyl stilbestrol, which was discontinued on December 9, 1946. FSH assay by the non-dialysis method in September 1947 was less than 96 m.u. per day; but by the dialysis method in October 1947, was more than 13 m.u. per day. In November 1948, FSH assay by the non-dialysis method was greater than 96 m.u. but less than 192 m.u. per day. 17-Ketosteroids in March 1948 were 5.0 mg. per day. *Testicular biopsy* (47-4285). In November 1947, the testis showed complete tubular sclerosis and interstitial fibrosis with no apparent recovery of tubules or Leydig cells.

Group B. Conditions with Normal FSH Excretion

(a) With endocrine disturbance

iv. *Lack of LH secretion?*

Case 39 (U-396128). (Reported as first patient by Lerman and Means (98).) This man, who was 46 years old in 1946, had bullet fragments in the pituitary area since the age of 15 years. *Treatment.* None before biopsy. *Laboratory findings.* Urinary 17-ketosteroids in 1943 were 3.2 mg. per day and in 1945, during omission of treatment, were 0.5 mg. per day. FSH assay in March 1943 by the dialysis method was greater than 3 m.u. per day but was not tested at higher levels. Two assays in 1948 were greater than 6.5 m.u.

but less than 13 m.u. per day. *Testicular biopsy* (43-2565). The right testis in April 1943 showed nearly normal tubules with a few sperm and no tunica propria thickening. No Leydig cells could be recognized (Fig. 8). *Exploration.* No craniotomy.

Case 41 (U-288692). (Reported as Case 26 by Klinefelter, Albright and Griswold (29).) This English salesman with 7 children was 55 years old in 1941. Development was normal but he had been weak since 1935. He had impaired vision and loss of libido since 1939. *Examination.* His height was 68 in. and weight 175 lbs. Axillary hair was absent and pubic hair was scant. Beard, phallus, testes, prostate, and breasts were normal. He had bilateral temporal hemianopsia. *Treatment.* Deep X-ray therapy to the pituitary in March 1941 and October 1941, and moderate amounts of androgen from September 24 to November 29, 1941. *Laboratory findings.* X-ray examination showed an eroded sella. The B.M.R. was −17 per cent and there was evidence of pernicious anemia. Semen was not studied. Urinary 17-ketosteroids before therapy were 3.5 mg. per day. FSH assay before therapy in 1941 by the non-dialysis method was less than 10 m.u. per 100 cc. Later, during testosterone therapy in 1942, assays by the dialysis method on 2 occasions showed more than 6.5 m.u. per day; and in 1944, on 2 occasions, were more than 26 m.u. but less than 52 m.u. per day. It is of interest that an assay in December 1948 showed less than 6.5 m.u. per day. *Testicular biopsy* (41-7390). The right testis, on December 1, 1941, showed nearly normally active tubules with a few sperm, slight thickening of the tunica propria and no Leydig cells (essentially like the histologic section shown in Figure 8). *Exploration.* No craniotomy.

v. *Acromegaly*

Case 43 (U-380940). The patient was a single, Irish laborer who was 19 years old in 1943. He had matured sexually at the normal age. He had had signs of acromegaly and headache since about 1941. *Examination.* His height was 72½ in., span 70 in., and weight 168 lbs. Hair, phallus, testes, prostate, breasts, voice and visual fields were normal. *Treatment.* No endocrine therapy before first biopsy. *Laboratory findings.* X-ray examination showed an enlarged sella and erosion into the sphenoid sinus. Serum phosphorus averaged 5.4 mg. per cent. The B.M.R. was −2 per cent. Semen was not studied. Urinary 17-ketosteroids (5 tests) averaged 10.1 mg. per day. FSH assay by the dialysis method in March 1943 was greater than 13 m.u. per day, and in April 1943 was greater than 26 m.u. but less than 52 m.u. per day. *Testicular biopsy* (43-2171). The left testis on April 15, 1943, showed nearly normal tubules without thickening of the tunica propria. There was a small number of typical Leydig cells. *Exploration.* No craniotomy.

(b) Without endocrine disturbance

vi. *Arrest of maturation in spermatogenesis*

Case 51 (U-501001). In 1945, this 33-year-old, English, naval officer had been married for seven years but had no children. He had normal potency and had not been exposed to X-rays. His wife was normal. *Examination.* His height was 67 in. and weight 132 lbs. Hair distribution, phallus, testes, breasts and voice were normal. His prostate was small. *Treatment.* No previous endocrine therapy. *Laboratory findings.* The B.M.R. was −19 per cent. Ten sperm counts showed occasional sperm, a few of which were motile. Urinary 17-ketosteroids were 8.3 mg. per day. FSH assay by the dialysis method in July 1945 was greater than 13 m.u. but less than 104 m.u. per day, and by the non-dialysis method in July 1948 was greater than 48 m.u. but less than 96 m.u. per day. *Testicular biopsy* (45-5721). The right testis in August 1945 showed normal spermatogenic activity up to

stage of spermatocytes, but there were only rare spermatids and sperm. There was no tunica propria thickening and the Leydig cells were normal.

Case 53 (Private). An English clerk, married for five years but having no children, was 31 years old in 1941. Two brothers had children, but another brother, Case 52, was also sterile. He had normal potency early in marriage but it had decreased recently. He had not been exposed to X-rays. His wife was normal. *Examination.* His height was 67 in. and weight 160 lbs. Hair distribution, breasts and voice were normal. Phallus and prostate were somewhat small. Large bilateral hydroceles obscured the testes. *Treatment.* None prior to biopsy; but chorionic and other gonadotropins from December 1941 to April 1942. *Laboratory findings.* The B.M.R. was −2 per cent. Semen repeatedly showed no sperm. Urinary 17-ketosteroids (6 tests) averaged 6.5 mg. per day. FSH assay by the dialysis method in September 1947 was less than 6.5 m.u. However, another test in May 1948 showed more than 13 m.u. but less than 52 m.u. per day. *Testicular biopsy* (41-6671). Both testes in November 1941 showed normal spermatogonia throughout, but a slightly decreased number of spermatocytes with a sudden arrest at spermatid stage, so that no mature sperm were present. Tunica propria and Leydig cells were normal (Fig. 9). (This case was previously reported (23), Case 5.) In neither Case 53 nor Case 52 have the patients shown sperm in ejaculates in the seven years since the original examinaition.

vii. *Hypospermatogenesis*

Case 54 (U-464624). In 1944, this 26-year-old Hebrew had been married for three years but had no children. He had normal potency and his wife was normal. *Examination.* His height was 68 in. and weight 175 lbs. His physique was entirely normal. *Treatment.* No previous endocrine therapy. *Laboratory findings.* Two semen analyses showed no sperm. Urinary 17-ketosteroids were 12.2 mg. per day. FSH assay by the dialysis method in August 1944 was greater than 13 m.u. but less than 104 m.u. per day. *Testicular biopsy* (44-6228). Both testes in September, 1944 showed a variable but generally marked decrease of spermatogenic activity at all stages. There was sloughing of immature forms into the lumina. Occasional tubules showed an apparent arrest at the spermatocyte stage, but Figure 3 of a previous paper (23) was not representative of the majority of tubules. Tunica propria and Leydig cells were normal.

Case 60 (Private.) In 1943, this 30-year-old Italian machinist had been married for three years but had no children. He was normally potent until one year before study. There was no history of X-ray exposure. His wife was normal. *Examination.* His height was 66½ in. and weight 160 lbs. His physique was normal except for left varicocele. *Treatment.* No endocrine therapy prior to biopsy. *Laboratory findings.* The B.M.R. was −2 per cent. Four sperm counts varied from 2 per h.p.f. to 16 million per cc. Urinary 17-ketosteroids were 8.5 mg. per day. FSH assay by the dialysis method in October 1943 was greater than 13 m.u. but less than 104 m.u. per day. *Testicular biopsy* (43-6406). The right testis in October 1943 showed variable but generally decreased spermatogenic activity, though spermatogonia were fairly numerous. Frequently there was sloughing of spermatocytes into the lumina, and mature sperm were rarely seen. There was no thickening of the tunica propria. Leydig cells were rather numerous (Fig. 10).

viii. *Oligospermia with normal testicular histology*

Case 70 (Private). In 1947, this 29-year-old English engineer had been married for seven years, without children. Potency was normal. His wife was normal but had had a presumed miscarriage in May 1946. *Examination.* His height was 70 in. and weight 165 lbs. His physique was normal, including the testes. *Treatment.* No previous endocrine

therapy. *Laboratory findings.* The B.M.R. was −16 per cent. In four seminal examinations the sperm counts varied from 38 million to 60 million per cc., motility from 50 to 60 per cent, and abnormal forms were approximately 50 per cent. Urinary 17-ketosteroids were 14.2 mg. per day. FSH assay in April 1947 by the dialysis method was greater than 13 m.u. but less than 52 m.u. per day, and in July 1948 by the non-dialysis method, less than 48 m.u. per day. *Testicular biopsy* (47-3393). The right testis in April 1947 showed many normal tubules containing sperm while other tubules were inactive with minor degenerative changes in the Sertoli cells and tunica propria. This variability possibly exceeded that normally seen. The Leydig cells were normal. *Follow-up note.* During management with diet, exercise and thyroid, 3 later seminal examinations showed sperm counts which varied between 62 and 94 million per cc. with only 20 per cent abnormal forms. No pregnancy has ensued.

Case 75 (U-580525). In 1947, this 31-year-old English physician had been married for eighteen months but had no children. He had had hepatitis in 1944. He had normal potency. His wife had anovulatory cycles. *Examination.* His height was 62 in. and weight 125 lbs. His physique was normal, including the testes. *Treatment.* No previous endocrine therapy. *Laboratory findings.* Three sperm counts varied from 32 million per cc. to 15 per h.p.f. Urinary 17-ketosteroids were 10.4 mg. per day. FSH assay by the dialysis method in July 1947 was greater than 6.5 m.u. but less than 13 m.u. per day. *Testicular biopsy* (47-5339). The right testis in June 1947 appeared normal in all respects.

Group C. Conditions with High FSH Excretion

ix. *Absence of germ cells*

Case 79 (U-557686). In 1946, this 36-year-old Irish office manager had been married for seven years and had no children. He had normal potency and had not been exposed to X-rays. His wife was normal. *Examination.* His height was 67 in. and weight 180 lbs. The phallus was normal. Both testes were within lower limits of normal. The prostate was slightly small. The breasts were prominent but only fat tissue was palpable. *Treatment.* No previous endocrine therapy. *Laboratory findings.* Semen on 2 occasions showed no sperm. Urinary 17-ketosteroids were 5.9 mg. per day. FSH assay in October 1947 by the dialysis method was greater than 13 m.u. but less than 52 m.u. per day, and in March 1949 by the non-dialysis method was greater than 192 m.u. per day. *Testicular biopsy* (46-9355). Both testes in December 1946 showed tubules with no germ cells but apparently normal Sertoli cells. There was no tunica propria thickening but slight peritubular proliferation and moderate increase of Leydig cells. (The histologic section resembled that shown in Figure 11.)

Case 80 (U-580508). In 1947, this 30-year-old Armenian truck driver had been married for eighteen months but his wife had not become pregnant. Potency was normal but diminished in recent months. There was no history of X-ray exposure. His wife was said to be normal. *Examination.* Hair distribution, breasts and voice were normal. Phallus and testes were slightly small. *Treatment.* No previous endocrine therapy. *Laboratory findings.* Semen on 2 occasions showed no sperm. Urinary 17-ketosteroids were 6.4 mg. per day. FSH assay in February 1948 by the dialysis method was greater than 13 m.u. but less than 104 m.u. per day; and in April 1949 by the non-dialysis method was greater than 96 m.u. but doubtful at 192 m.u. per day. *Testicular biopsy* (47-5337). The right testis in June 1947 showed absence of germ cells, normal Sertoli cells, normal tunica propria and normal numbers of Leydig cells.

Case 82 (Reported in a previous publication (23)—Case 8 and Figure 6.) This man

was 24 years old in 1943. *Testicular biopsy* (43-5300). The right testis in August 1943 showed no germ cells except in a single normal tubule, and there was an increased mass of Leydig cells. *Subsequent data.* Urinary 17-ketosteroids in October 1947 were 12.1 mg. per day. FSH assay in October 1947 by the dialysis method was more than 13 m.u. but less than 52 m.u. per day; and in August 1948 by the non-dialysis method was greater than 48 m.u. but less than 96 m.u. per day. The highest sperm count observed was 5 million per cc. The wife had a normal baby in May 1949.

Case 83 (Private). In 1941, this 29-year-old Lithuanian psychologist had been married for two years but had no children. He had a skull fracture in childhood without complications. He showed normal development and potency and there had been no exposure to X-rays. His wife was normal. *Examination.* His height was 70½ in. and weight 149 lbs. His physique was normal, except for slightly small testes and a large left varicocele. *Treatment.* None until June 1941, when desiccated thyroid and chorionic gonadotropin were given until September 1941. From October 1941 to January 1942 he was given testosterone, but he had had none since then. *Laboratory findings.* More than 3 semen examinations showed no sperm. Urinary 17-ketosteroids in 1941 (4 tests) averaged 3.0 mg. per day. FSH assay by the non-dialysis method in June 1941 was less than 10 m.u. per 100 cc.; in April 1942 by the dialysis method more than 6.5 m.u. per day; in December 1947 by the dialysis method more than 13 m.u. but less than 104 m.u. per day; and in April 1949 by the non-dialysis method more than 192 m.u. per day. *Testicular biopsy* (41-2139). The right testis in April 1941 showed no germ cells, normal Sertoli cells, normal tunica propria and a moderately increased number of Leydig cells. (*See* Figure 8 in the paper of Simmons and Sniffen (23) and Figure 2 in the paper by Simmons (27).) *Repeat biopsy* (41-5643). The left testis in September 1941 was similar.

Case 85 (U-488949). In 1945, this 38-year-old, English, teacher had been married for thirteen years and had no children. He had normal potency and there was no history of X-ray exposure. His wife was normal. *Examination.* His height was 73 in. and weight 180 lbs. He had a normal physique except for small testes. *Treatment.* No previous endocrine therapy. *Laboratory findings.* X-ray examination showed a normal skull. Semen showed no sperm on 5 examinations. Urinary 17-ketosteroids (2 tests) averaged 5.6 mg. per day. FSH assay in May 1945 by the dialysis method was more than 104 m.u. per day; in January, 1946 by the non-dialysis method was greater than 96 m.u. but less than 192 m.u. per day; and in November 1947 was greater than 192 m.u. but less than 288 m.u. per day. *Testicular biopsy* (45-3132). The right testis in May 1945 showed absence of germ cells, normal Sertoli cells, slight thickening of tunica propria, slight peritubular proliferation and markedly increased numbers of Leydig cells.

Case 88 (U-344876). In 1942, this 30-year-old, Italian, bottle maker had been married for two years and had no children. He had normal potency and there was no history of X-ray exposure. His wife was normal. *Examination.* His height was 65 in. and weight 140 lbs. He had a normal physique except that both testes were about one-third normal size. *Treatment.* No previous endocrine therapy. *Laboratory findings.* No sperm in semen on 3 examinations. Urinary 17-ketosteroids were 8.8 mg. per day. FSH assay in February. 1942 by the non-dialysis method was greater than 10 m.u. but less than 30 m.u. per 100 cc.; and in December, 1948 greater than 96 m.u. but less than 192 m.u. per day. *Testicular biopsy* (42-213). The left testis in January 1942 showed absence of germ cells, normal Sertoli cells, normal tunica propria and moderately increased numbers of typical Leydig cells (Fig. 11).

Case 91 (Private). In 1945, this 26-year-old English physicist had been married for five years without children. Potency was normal. He had been a cyclotron worker during

the previous five years but he had had no X-ray therapy. His wife was normal. *Examination.* His height was 74 in., span 76 in. and weight 185 lbs. Hair distribution, breasts and voice were normal. Phallus and prostate were small. Both testes were half normal size. *Treatment.* No previous endocrine therapy. *Laboratory findings.* Semen on 2 occasions showed no sperm. Urinary 17-ketosteroids were 9.4 mg. per day. FSH assay by the dialysis method in April 1945 was more than 13 m.u. but less than 104 m.u. per day. *Testicular biopsy* (45-2119). Both testes in March 1945 showed no germ cells, normal Sertoli cells, normal tunica propria and normal numbers of Leydig cells.

Case 92 (Private). In 1942, this 27-year-old man had been married for three years and had no children. He was a salesman of X-ray machines without other exposure to radiation. Potency was apparently normal until appendectomy shortly after marriage. His wife was normal. *Examination.* His height was 69 in. and weight 177 lbs. His physique was normal, including size of testes. *Treatment.* No endocrine therapy before September 1942. During the following year he was given intensive injections of chorionic and other gonadotropins. *Laboratory findings.* The B.M.R. was −3 per cent. Semen repeatedly showed no sperm. Urinary 17-ketosteroids (2 tests) averaged 11.7 mg. per day. FSH assay by the dialysis method in August 1942 was greater than 26 m.u. but less than 52 m.u. per day. However, by the non-dialysis method in January 1945, assay was greater than 192 m.u. but less than 384 m.u. per day; and in October 1947, more than 96 m.u. but less than 192 m.u. per day. *Testicular biopsy* (42-5062). The right testis in July 1942 showed no germ cells, normal Sertoli cells, normal tunica propria and normal numbers of Leydig cells (*see* Case 4 and Figure 4 in a previous paper (23)). *Repeat biopsy* (43-4625). The right testis in July 1943 showed no differences.

Case 94 (U-479601). In 1945, this 31-year-old English banker had been married five years without children. He was normally developed and was potent. When 15 years old he received a course of roentgen therapy to the perineal-scrotal region for eczema. His wife was normal. *Examination.* His height was 69 in., and weight 150 lbs. Hair, breasts, voice and phallus were normal. Both testes were small and soft and the prostate was small. *Treatment.* Methyltestosterone for six months in 1942. Thyroid therapy during 1944–45. *Laboratory findings.* The B.M.R. was −23 per cent. Semen counts elsewhere were 2 million per cc., and by our examination 3–6 per h.p.f. Urinary 17-ketosteroids in 1945 were 11.2 mg. per day. FSH assay by the dialysis method in February 1945 was greater than 104 m.u., and the same result was found in May 1948. *Testicular biopsy* (45-1141). Both testes in February 1945 showed complete absence of germ cells throughout the specimens but normal Sertoli cells, slight thickening of the tunica propria and normal numbers of Leydig cells.

Case 95 (U-588722). In 1947, this 22-year-old, English, student-veteran had been married for two and a half years and had no children. His left testis had been undescended since birth; the right had always been scrotal. He had normal potency, and there was no history of X-ray exposure. His wife was normal. *Examination.* His height was 72 in., span 72 in., and weight 185 lbs. His physique was normal except that the left testis was in the inguinal canal and the right scrotal testis was slightly small. *Treatment.* One year before study he received a few injections of pregnant mare serum. *Laboratory findings.* The B.M.R. was −10 per cent. He was reported elsewhere to have had 5 million sperm per cc. in 1946. By our examination, 2 specimens in 1947 showed no sperm. Urinary 17-ketosteroids were 5.2 mg. per day. FSH assay by the dialysis method in September 1947 was greater than 13 m.u. but less than 52 m.u. per day. *Testicular biopsy* (47-7026). The right scrotal testis in September 1947 showed no germ cells, normal Sertoli cells, normal tunica propria and a slight increase of typical Leydig cells (similar to the histologic sec-

tion shown in Figure 11). The left retained testis showed an identical picture, except that many of the Leydig cells had a foamy degeneration of their cytoplasm.

Case 96 (U-587174). This patient was a single, Hebrew student who was 19 years old in 1947. The left testis was not in the scrotum in childhood; the right had always been scrotal. He was an obese boy and received frequent injections of chorionic gonadotropin elsewhere between the ages of 9 and 12 years. Pubertal changes began at 10 years and proceeded normally. He had erections, but he stated that the left testis did not descend until the age of 14 years. Large breasts were also noted at 14 years. He had never been exposed to X-rays. *Examination.* His height was 64½ in., span 69 in., and weight 189 lbs. Hair distribution, voice, phallus and prostate were normal. Both testes were normal in size and position. There was marked enlargement of the breasts, which showed, on subsequent histologic examination, isolated ducts in very dense collagenous tissue. *Treatment.* No endocrine therapy since the age of 12 years. *Laboratory findings.* X-ray examination showed a normal sella. Semen was not studied. Urinary 17-ketosteroids were 10.2 mg. per day. FSH assay by the non-dialysis method in September 1947 was less than 96 m.u. per day, and by the dialysis method in November 1947 was greater than 13 m.u. per day. *Testicular biopsy* (47-6743). The right testis in August 1947 showed no germ cells except in a single tubule which showed active spermiogenesis (Fig. 12). Elsewhere tubules were of nearly normal size but contained only well preserved Sertoli cells. Tunica propria and Leydig cells were essentially normal, and there was a slight excess of interstitial fibrocytes.

x. *Sclerosing tubular degeneration*

(*a*) *with little "eunuchoidism" and with gynecomastia*

Case 97 (U-141135). (Reported as Case 6 by Klinefelter, Reifenstein and Albright (10).)

Case 98 (U-119837). (Reported as Case 3 by same authors (10), and as Case 134 by Fraser and co-workers (28).)

Case 99 (U-12047). (Reported as Case 8 by Klinefelter and co-workers (10).)

Case 102 (U-344755). (Reported as Case 1 by same authors (10).)

Case 105 (U-425896). This single, Syrian pharmacist was 23 years old in 1943. He complained of large breasts, small testes and impotence. The patient in Case 106 was his brother and in Case 104, his maternal uncle. Breast enlargement was present in childhood. Puberty commenced at the age of 14 years; he had erections without ejaculations. *Examination.* His height was 67 in. and weight 169 lbs. He had moderate acne, no beard, and a moderate amount of axillary and pubic hair. He had a normal-sized phallus with slight hypospadias, very small scrotal testes, and a normal prostate. His voice was deep and he had large, pendulous breasts. *Treatment.* In 1939 he had received about 10,000 units of chorionic gonadotropin; and in 1942 testosterone pellets totalling 300 mg. were implanted at another hospital. No therapy in 1943. *Laboratory findings.* Elsewhere, urinary estrogens were reported absent in 1939. The semen showed no sperm. Urinary 17-ketosteroids in 1943 (3 tests) averaged 14.7 mg. per day. FSH assay in October 1943 by the non-dialysis method was less than 96 m.u. per day, and in December 1943 by the dialysis method was greater than 13 m.u. but less than 26 m.u. per day. *Testicular biopsy* (43-6708). The testis in October 1943 showed almost complete tubular sclerosis, but a few remaining tubules showed degenerating germ cells without sperm, atrophic Sertoli cells and thickened tunica propria. Here and there a small immature type of tubule was seen and these were involved in the sclerosis. There were large masses of Leydig cells, all of which appeared to have a dense homogeneous cytoplasm. *Subsequent course.* His

breasts, which were removed in January 1944, showed ductal hyperplasia and periductal fibrous proliferation. He was treated intensively with chorionic gonadotropin and testosterone from February 18, 1944 to August 3, 1944. Potency and hair growth improved but testicular biopsies in March, May and July 1944 did not reveal any changes. FSH assay in October 1944 by the dialysis method was greater than 52 m.u. but less than 78 m.u. per day. After nearly eighteen months without treatment, FSH assay in January 1946 by the dialysis method was greater than 104 m.u. per day. This case has been reported by Reifenstein (49).

Case 110 (U-317255). (Reported as Case 7 by Klinefelter, Reifenstein and Albright (10).) Urinary FSH assay by the non-dialysis method in July 1941 was greater than 10 m.u. per 100 cc., and in Mar. 1942 greater than 270 m.u. but less than 450 m.u. per day. *Testicular biopsy* (41-5221). The right testis in August 1941 showed some areas with relatively normal tubules except for thickening of tunica propria, whereas others showed more marked sclerosis with atrophy of tubular cells (Fig. 13). The Leydig cells appeared increased in numbers, usually showing a dark homogeneous cytoplasm but occasionally cellular disintegration.

Case 111 (U-346183). (The details of this complicated history were previously reported as Case 9 by Simmons and Sniffen (23).) The present diagnosis is favored by the course and present findings including those of a testicular biopsy (42-3097), which showed variable areas of nearly normal and degenerating tubules with rather prominent Leydig cells. On 3 occasions in 1942 and another in 1945, FSH assays by the non-dialysis method were abnormally high.

(*b*) *With little "eunuchoidism" and without gynecomastia*

Case 114 (U-444014). This single, Slavic farmer was 29 years old in 1944. He was referred because of small testes. Sexual changes commenced at about 14 years. He had ejaculations but never had intercourse. He shaved daily. *Examination.* His height was 67 in., span 71 in. and weight 145 lbs. His beard was scant but axillary and pubic hair phallus, prostate, breasts and voice were normal. Each testis was only 2 cm. in length. *Treatment.* No endocrine therapy prior to study. *Laboratory findings.* The B.M.R. was −14 per cent. Seminal fluid examinations twice showed no sperm. X-ray examination showed that the wrist epiphyses were closed. Urinary 17-ketosteroids (2 tests) averaged 12.3 mg. per day. FSH assay in November 1944 by the dialysis method was greater than 13 m.u. but less than 104 m.u. per day, but in January 1945 by the non-dialysis method it was greater than 384 m.u. per day. *Testicular biopsy* (44-7780). The right testis in November 1944 showed many sclerosed tubules, and large masses of Leydig cells with only sliight evidence of cytologic abnormalities (Fig. 14). *Subsequent course.* Eight weeks of testosterone therapy resulted in frequent erections but no improvement in testicular histology (biopsy 45-1628).

(*c*) *With marked "eunuchoidism" and with gynecomastia*

Case 118 (U-147829). (Reported as Case 2 by Klinefelter, Reifenstein and Albright (10).)

Case 119 (U-296608). (Reported as Case 5 by same authors. (10).)

(*e*) *Doubtful examples*

Case 126 (U-566196). The patient was an English, reform school inmate, who was 17 years old in 1947. Breast enlargement began at 9 years, and pubic hair and erections oc-

curred at 13 years. *Examination.* His height was 68 in., span 66 in. and weight 140 lbs. There was no beard or axillary hair. Pubic hair, phallus and voice were normal. Both testes were in normal position and of nearly full adult size. The prostate was small and there was moderate enlargement of both breasts. *Treatment.* None prior to study. *Laboratory studies.* Semen was not studied. Urinary 17-ketosteroids were 7.0 mg. per day. FSH assay by the non-dialysis method in March 1947 was greater than 106 m.u., but less than 212 m.u. per day. Removed breasts showed marked ductal epithelial hyperplasia and loose periductal fibrous proliferation. *Testicular biopsy* (47-2602). The right testis in April 1947 showed many normal adult tubules but others with slight degenerative changes of Sertoli cells and germ cells. There was no thickening of the tunica propria. The Leydig cells seemed slightly more numerous than normal and an unusual number showed degenerative changes.

xi. *Absence of testes*

Case 127 (U-84187). A Hebrew lawyer, single, was 26 years old in 1943. Since birth, there had been no scrotal testes. When 8 years old, at the Children's Hospital, Dr. Ladd explored the canals and scrotum, and he isolated vasa ending in rudimentary gonads. Some pubic hair grew, but no other secondary sexual development occurred till treatment in 1940. *Examination.* His height was 69 in. and weight 193 lbs. He was obese and his span exceeded his height. In 1943 he had no beard, moderate pubic hair, and a small phallus; later, after further therapy, body hair, phallus and voice became normal. *Treatment.* In January 1940 testosterone propionate was started in a dose of 25 mg. three times weekly; this was changed in July 1940 to methyltestosterone, 10 mg. daily, until September 1941. He had no further therapy except desiccated thyroid until 1944. *Laboratory findings.* X-ray examination showed a normal skull. Urinary 17-ketosteroids during a period of no treatment in 1943 (2 tests) averaged 18.0 mg. per day. FSH assay, without therapy, in November 1943 by the non-dialysis method was greater than 192 m.u. per day; and twice during January 1944 was greater than 576 m.u. but less than 868 m.u. per day. *Testicular biopsy* not done. (This case was mentioned by Reifenstein (78).)

Case 129 (U-127979). (Reported by Fraser and coworkers (28) as their Case 69.)

Case 130 (U-18281). This single, Italian law student was 21 years old in 1942. Swollen testes followed birth trauma, but there were no palpable scrotal testes thereafter. At 12 years, bilateral inguinal and retroperitoneal exploration revealed no gonads. At 16 years he had some pubic hair and a small adult-sized penis. No ejaculations occurred. *Examination.* His height was 71½ in., span 76 in., and weight 157 lbs. He had no beard but had some axillary and pubic hair without abdominal extension, and with a poor growth rate. The phallus was within the lower limits of normal size. The prostate was small. The breasts were slightly enlarged, but biopsy showed fat only. *Treatment.* He was given a few chorionic gonadotropin injections in 1937 and more in 1940. Since November 1942 he received adequate testosterone therapy. *Laboratory findings.* X-ray examination showed delay in the union of the epiphyses of the hand. Urinary 17-ketosteroids during a period of no treatment in 1940 (4 tests) averaged 8.8 mg. per day. FSH assay by the non-dialysis method on 2 occasions during January 1940 was greater than 10 m.u. per 100 cc.; and in April 1942 was greater than 30 m.u. but less than 50 m.u. per 100 cc. By the same method in June 1943 it was greater than 192 m.u. per day; in February 1944, greater than 288 m.u. but less than 576 m.u. per day; and in November 1947, greater than 192 m.u. per day. *Testicular biopsy* (42-3793). Scrotal exploration revealed one tiny nubbin, but histologic examination showed fibrous tissue without testicular elements. (Fraser and co-workers (28) reported this as their Case 67.)

xii. *Normal testicular histology (? climacteric)*

Case 132 (U-406089). In 1944, this 55-year-old German watchman had been married for twenty-three years and had 2 children. He complained of loss of potency, dizzy spells and anxiety since 1943. *Examination.* His height was 68½ in. and weight 180 lbs. His physique was entirely normal. *Treatment.* From March to June 1944 he was given methyltestosterone, 10 mg. or 20 mg. daily, with improvement. No further therapy was given till 1945. *Laboratory findings.* X-ray examination showed a normal sella. Semen was not studied. Urinary 17-ketosteroids (2 tests) during a period of no treatment averaged 4.7 mg. per day. FSH assays by the non-dialysis method in August 1944 and in November 1944 were greater than 192 m.u. per day; and a sample in December 1947 showed more than 96 m.u. but less than 192 m.u. per day. *Testicular biopsy* (44-6747). The left testis in October 1944 showed essentially normal structure in all respects with active spermatogenesis and only scattered sclerotic tubules compatible with patient's age.

Case 136 (Private). In 1943, this 30-year-old English, teacher, had been married for four years and had no children. Pubertal changes occurred at 14 years. He was hospitalized for tuberculosis between the ages of 18 and 20 years. He had normal potency and no hot flashes. His wife was normal. *Examination.* His height was 70 in. and weight 135 lbs. His secondary sex characteristics and testes were normal. *Treatment.* After biopsy in 1943, he was given six weeks' chorionic gonadotropin therapy of 100 units daily; and in December 1945 he was given eight weeks of therapy with 200 units daily. *Laboratory findings.* The initial sperm count was less than 1 million per cc., but after therapy, counts of 30–69 million per cc. were found in 1943. Urinary 17-ketosteroids in 1946 were 7.0 mg. per day. FSH assay in March 1946 by the dialysis method was greater than 104 m.u. per day, and in September 1947 by the non-dialysis method greater than 192 m.u. per day. *Testicular biopsy* (43-4003). The right testis in June 1943 appeared normal in all respects.

xiii. *Bilateral mumps orchitis*

Case 137 (U-427220). (Reported as Case 1 by Simmons and Sniffen (23).) This patient was 31 years old in 1943.

Case 138 (U-591027). This Greek patient was 50 years old in 1948. He had had mumps with bilateral orchitis at the age of 25 years. He had been married for about fifteen years without children, but with normal potency. He had suffered from renal calculi, bone pains and weakness for several years. Relevant examinations substantiated the diagnosis of hyperparathyroidism and the hypertrophic glands were removed in September 1947. He also had chronic lymphatic leukemia with splenomegaly but he was not given therapy for this. *Examination.* Hair distribution, voice, breasts, prostate and phallus were normal but the testes were atrophic. *Treatment.* No endocrine therapy. *Laboratory studies.* X-ray examination showed a normal sella. Semen was not studied. Urinary 17-ketosteroids were 2.0 mg. per day. FSH assay in September 1947 by the non-dialysis method was greater than 96 m.u. per day. *Testicular biopsy* (48-967). Both testes in February 1948 were as described in Table 14 and as illustrated in Figure 15.

Case 139 (Private). In 1947, this 31-year-old English college student had been married for three years and had 1 child. Thereafter, in July 1946, he suffered from mumps with bilateral orchitis. *Examination.* His physique was normal, including the size of both testes. *Treatment.* None prior to study, except desiccated thyroid. *Laboratory findings.* The sperm count in March 1947 was 17 million per cc. with 95 per cent motile, but semen in April 1947 showed only rare sperm. Urinary 17-ketosteroids were 8.9 mg. per day. FSH assay in December 1947 by the dialysis method was greater than 104 m.u. per day;

and in August 1948 by the non-dialysis method, was greater than 192 m.u. per day. *Testicular biopsy* (47-6611). Both testes in August 1947 were as described in Table 14.

xiv. *Myotonic dystrophy*

Case 140 (U-210087). This English, retired postman was 63 years old in 1947. He had three children. A son had myotonia. The patient had weakness and impotency since 1938. *Examination.* He showed bilateral cataracts, myotonia and wasting. The secondary sex characteristics were normal but each testis was only 2 cm. long. *Treatment.* He received a short course testosterone propionate in 1939 but none since. *Laboratory findings.* Semen was not studied. Urinary 17-ketosteroids (2 tests) in 1947 averaged 3.5 mg. per day. FSH assay in July 1947 by the non-dialysis method was less than 96 m.u. per day, and by the dialysis method less than 6.5 m.u. per day. *Testicular biopsy* (47-4097). The left testis in May 1947 was as described in Table 14.

xv. *Bilateral cryptorchidism*

Case 141 (U-469663). This patient was 17 years old in 1948. His height was 59 in., span 59 in. and weight 79 lbs. He was sexually immature. X-ray examination showed a normal skull. FSH assay in December 1947 by the dialysis method was greater than 52 m.u. per day; and by the non-dialysis method, greater than 192 m.u. per day. Tissue was removed from each testis for biopsy in March 1948. Other details are presented in Table 14 and Figure 16.

STUDIES ON THE EFFECT OF EPINEPHRINE ON THE PITUITARY-ADRENOCORTICAL SYSTEM*

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IN 1908, Babes and Jonescu observed that conditions of stress in man and laboratory animals were associated with the depletion of suprarenal lipid. Multiple intravenous injections of epinephrine produced a similar depletion, accompanied by adrenal hypertrophy in rabbits (1). Vogt later demonstrated that following the administration of epinephrine, cortical hormones could be detected in the adrenal venous blood of cats and dogs (2). Long and Fry (3) studied this response of the adrenal cortex to epinephrine, using the assay of Sayers (4) for adrenal corticotropic hormone (ACTH). They described a significant ACTH release in response to injected epinephrine in normal rats; on the other hand, hypophysectomized rats failed to show this response, in spite of the fact that their adrenal cortices were maintained with exogenous pituitary hormone injections. From these data Long postulated that the effect of epinephrine on the adrenal cortex was mediated by the pituitary. Vogt subsequently reported similar differences between hypophysectomized and normal rats in response to epinephrine (5). This work provided additional support to the postulation of the mediatory role of the pituitary in the adrenal cortical changes induced by epinephrine.

Selye pointed out the physiologic integration of neuro-endocrine responses to stress in his description of the general adaptation syndrome (6). He showed that adaptation is largely dependent upon the initial sympathetic reaction of the body to nonspecific stimuli. This reaction is then succeeded by the mobilization of the pituitary and adrenal cortical hormones. Epinephrine release affords the immediate protection, while the adrenal cortical hormones provide more sustained effects. The sequence of these reactions suggests a coordinating mechanism. Although epinephrine appears to be an important link in this integrated system, it has not been demonstrated conclusively to act upon the anterior pituitary, nor has it

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been shown to be the *only* agent concerned with the activation of pituitary ACTH secretion.

The appearance of eosinopenia under conditions of stress has been known to clinicians for many years. More recently, Dalton and Selye demonstrated in rats that an eosinopenia constitutes a characteristic part of the alarm reaction (7). Thorn *et al.* (8), have shown that depression in the number of circulating eosinophils follows the intramuscular administration of ACTH in man. This depression is also noted following the intravenous administration of 17-hydroxycorticosterone and related 11, 17-oxysteroids of the adrenal cortex (9).

Although eosinopenia following epinephrine administration to experimental animals and man has been noted (10, 11) at no time has this change in eosinophils been related to adrenal cortical activity. In a preliminary note, Recant *et al.* showed that the epinephrine-induced eosinopenia in rat and man was dependent on pituitary-adrenal integrity(12).

In this paper, humoral factors affecting the number of circulating eosinophils have been investigated in rat, dog, and man. An attempt has been made to establish that a change in the level of eosinophils is a valid reflection of adrenocortical function. The relative importance of epinephrine in reactions to stress has been studied. Observations have been made on the mechanism of action of epinephrine on the pituitary-adrenocortical system. A simple clinical test for the integrity of the pituitary-adrenocortical system is presented on the basis of the data obtained.

METHODS

Experimental Subjects

Male and female albino rats of the Sprague-Dawley strain, weighing 200–260 grams, were studied. These included normal, adrenalectomized, demedullated, and hypophysectomized groups. The animals were fed Purina Dog Chow and allowed water ad libitum up to the time of the experimental period. During the period of observation, the animals were fasted.

Mongrel dogs, male and female, weighing 10–15 Kg. were used. The groups included normal, bilaterally adrenalectomized, hypophysectomized, anterior hypophysectomized, stalk-sectioned and sympathectomized animals. The diet consisted of ground horse meat $\frac{2}{3}$ and Ken-L Bisket (kibbled) $\frac{1}{3}$, amounting to $\frac{3}{4}$ to 1 pound a day. Water was allowed ad libitum. The animals were fasted overnight, prior to, and during the experimental procedures.

Tests were also carried out on normal human subjects, individuals with Addison's disease and patients with hypopituitarism. They were main-

tained on regular diets and fasted overnight, prior to, and during the experiments.

Operative Procedures

Rats:—Under ether anesthesia, adrenalectomy and adrenal demedullation were performed by the lumbodorsal approach. Splenectomy was carried out by the anterior approach. The adrenals and perirenal fat were removed. The adrenal gland was enucleated in the process of demedullation, leaving only a thin rim of capsule for the subsequent regeneration of the cortex. Hypophysectomy was carried out by the paratracheal route and the entire gland was removed under direct vision.[1] Hypophysectomized and adrenalectomized animals were maintained on 1 per cent saline drinking water. The adrenalectomized animals received implants of desoxycorticosterone acetate pellets. Spot autopsies were performed at the close of the experiments.

Dogs:—The procedures were carried out either under pentothal-induced intratracheal ether or intravenous nembutal anesthesia.

Adrenalectomy was performed in two stages: the right adrenal was removed first under nembutal anesthesia; the left adrenal was removed seven to forty days later under ether anesthesia. The operation was done transperitoneally through the bed of the resected twelfth or thirteenth rib. A 125 mg. desoxycorticosterone acetate pellet was implanted at the time of the second operation. During the second procedure, the animals received a 500 cc. infusion of a solution containing sodium chloride 3 Gm./L, potassium chloride 2.9 Gm./L, and hydrated disodium hydrogen phosphate 9 Gm./L. Postoperatively, the animals were maintained on a regular diet with the daily addition of 500 cc. of milk to which 10 Gm. of sucrose and 1–2 Gm. of salt had been added. This supplementary feeding was discontinued after two to three months.

Hypophysectomies were performed via the transbuccal and transtemporal approaches with intravenous nembutal anesthesia. The latter approach was used for the anterior hypophysectomies and stalk sections. In one of the stalk-sectioned dogs, a piece of polyethylene film was placed between the pituitary and the hypothalamus to prevent regrowth of the stalk blood vessels. The dogs were given the regular diet postoperatively.

Sympathectomies were performed in three stages, with one- to two-week intervals between each stage. The first two stages were carried out transthoracically and the incision was made in the sixth interspace. The sympathetic chain was removed from the stellate ganglion to L_1. The greater

[1] The authors are indebted to Dr. O. Greep of the Harvard Dental School for the rat hypophysectomies.

splanchnic was divided and removed and a silver clip was placed on the distal cut end opposite the first lumber vertebra. The third stage was done transperitoneally through a midline incision. The sympathetic chain was removed bilaterally from L_1 through L_4. The cut ends of the greater splanchnics, with their clips, were pulled through the diaphragm. They were then removed with a portion of the coeliac ganglion. The lesser and least splanchnics were likewise removed. The vagi were left intact. The dogs were placed on the regular diet postoperatively.

Hormonal Preparations

1. *Aqueous adrenal cortical extracts:*

A, B and C, represented three commercial preparations which were administered intraperitoneally to rats and intravenously to patients.[2]

2. *Adrenal steroids:*

Compounds E acetate,[3] F,[4] and desoxycorticosterone glucoside[5] were used. Compound E acetate was dissolved in warm absolute alcohol (25 mg. per cc.) and administered intravenously to dogs and patients. Small amounts were injected through the rubber tubing of a saline infusion at regular intervals over a one-hour period. This procedure was found necessary because of the marked insolubility of the material. Crystalline Compound F was given intraperitoneally to rats as a suspension in saline. It was dissolved in absolute alcohol, mixed with saline, and given intravenously or in 1 per cent procaine intramuscularly to human subjects. Crystalline, water-soluble, desoxycorticosterone glucoside was administered intravenously in saline to human subjects.

3. *Adrenocorticotropin:*

Pork pituitary ACTH (Armour Lot G-59703)[6] was administered intraperitoneally to rats and intramuscularly to dogs and patients. The material was a sterile powder, readily soluble in saline. The activity of this material

The authors are indebted:

[2] To Mr. F. Neldon of the Division of Research Development, Parke, Davis and Company for the ample provision of Eschatin and Adrenalin hydrochloride.

To Dr. D. Klein of Wilson and Company for the provision of Adrenal Cortex Extract.

[3] To Dr. Augustus Gibson, Merck and Company, Rahway, N. J. for the Compound E acetate.

[4] To Dr. M. H. Kuizenga, The Upjohn Company, Kalamazoo, Michigan, for Compound F and Upjohn's Adrenal Cortical Extract.

[5] To Dr. E. Oppenheimer, Ciba Pharmaceutical Products, Inc., Summit, N. J., for the desoxycorticosterone glucoside and the pellets of desoxycorticosterone acetate (Percorten).

[6] The authors gratefully acknowledge the ample supply of purified ACTH provided by Dr. John R. Mote, Armour Laboratories, Chicago, Ill.

was equivalent to 15.8±4 per cent of the Armour standard LA-1-A by the rat adrenal ascorbic acid assay of Sayers as modified by Munson. This standard will induce a 20 to 30 per cent fall in adrenal ascorbic acid when a dose of 4 gammas is administered intravenously to a hypophysectomized rat. Dosage refers to this standard throughout.

4. *Epinephrine hydrochloride:*

1:1000, was given intraperitoneally to rats and subcutaneously and intravenously in normal saline in varying dilutions to dogs and patients. Solutions were prepared freshly from a 1 cc. ampule.

Hematologic Methods

Blood samples in rats were obtained by sectioning the tip of the tail with a sharp scalpel and withdrawing the blood directly into the white counting pipette. Venous blood samples drawn into balanced oxalate were used in the dogs and human subjects. Total white blood counts, differential blood counts performed on 200–400 cells, and hematocrits were carried out by the routine methods. Eosinophil counts were performed according to a modification of the direct counting technique of Dunger (13).

Precautions for the exclusion of foreign particles in every step of the procedure are essential. Blood is drawn up to the 0.5 mark in a white cell pipette and diluted with special fluid to the 1.1 mark, shaken gently 50 times and no more, and plated immediately in a Fuchs-Rosenthal counting chamber of 0.2 mm. depth. After three minutes, the eosinophils have picked up the red stain. The cells are then counted under the low power of the microscope. Two chambers are set up for each count and thus four fields are counted. The calculation used is:

$$\frac{\text{average count per field} \times 100}{16} = \text{eosinophils per cu. mm.}$$

The diluting fluid is made up every ten days, stored in the cold, filtered daily before use, and prepared as follows:

Aqueous yellow eosine, 2%	2.5 cc.
Acetone	2.5 cc.
Distilled water to	50.0 cc.

Chemical Methods

Blood sugar was determined by the method of Folin and Wu (14). The Somogyi zinc precipitation (15) was used to eliminate nonfermentable reducing substances. Uric acid was determined by an adaptation of Archibald's[7] (16) modification of the method of Kern and Stransky (17). Cre-

[7] This method was made available by Dr. Reginald Archibald prior to publication and modified for urine analysis by one of the authors.

atinine was determined by the method of Folin and Wu (18). The 17-ketosteroid level was estimated by a modification of the method of Callow (19) and the 11 oxysteroid level by the colorimetric method of Talbot,[8] *et al.* (20).

OBSERVATIONS

The number of circulating eosinophils is greater in rat and dog than in man (Table 1). Adrenal insufficiency in man and dog is usually accom-

TABLE 1. ABSOLUTE EOSINOPHIL LEVELS IN RAT, DOG AND MAN
(No. per cu. mm.)

	No. of Cases	Mean	Range	No. of Cases	Mean	Range	No. of Cases	Mean	Range
Rat	12	1101	356–3090	12	1170	584–4240	12	894	624–1680
Dog	18	765	50–3000	6	3703	375–8310	4	1148	730–1600
Man	50	181	45–344	33	327	94–1840	13	184	83–544

panied by higher eosinophil levels than in control groups (Table 1). However, in man this difference is not statistically significant, perhaps because of the wide individual variations and the small sampling.

The variations in the initial level of circulating eosinophils in normal individuals suggests the existence of factors other than adrenal cortical hormones governing the regulation of the level. Allergic states with accom-

TABLE 2. CHANGES IN CIRCULATING EOSINOPHILS FOLLOWING THE SECOND STAGE OF A BILATERAL ADRENALECTOMY IN THE DOG

Dog	Date	Count	Dog	Date	Count	Dog	Date	Count
No. 99	Before operation		No. 91	Before operation	188	No. 126	Before operation	794
	Operation			*Operation*			*Operation*	
	5–12	0		4–28	0		6–21	350
	5–13	0		5–11	1980		6–22	780
	5–14	345		5–14	3450		6–23	850
	5–15	475		5–17	4230		6–24	836
	5–18	2000		5–21	2820		6–28	1060
	5–21	3000		5–22	3310		7–22	8310
				6–7	2660			
				8–4	3975			
				1–10	6375			

[8] We are indebted to Dr. N. Talbot and his laboratory for the 11-oxysteroid determinations.

panying eosinophilia, and bone marrow hypoplasia with eosinopenia are examples of situations in which nonadrenal factors appear to be of primary importance in the maintenance of the eosinophil levels. The importance of the adrenal cortex as one factor in the regulation of the level of circulating eosinophils is suggested by the significant rise in eosinophils following bilateral adrenalectomy in dogs (Table 2). This is further supported by the

TABLE 3. COMPARISON OF FASTING EOSINOPHIL LEVELS TAKEN APPROXIMATELY ONE YEAR APART IN PATIENTS WITH ADDISON'S DISEASE*

Patient	1947	1948
J.C.	113	119
M.H.	114	142
A.T.	117	147
E.C.	137	192
E.G.	140	176
M.T.	169	139
A.C.	210	151
M.C.	247	154
H.S.	259	288
N.M.	327	308
R.S.	288	506
M.F.	470	306
E.V.	562	519

* Patients treated with adrenal extract are not included.

relative constancy of the eosinophil level from year to year in patients with Addison's disease (Table 3). This stands in contrast to the large variation in the level of eosinophils commonly observed in normal individuals.

The Effect of Changes in the External Environment on the Circulating Eosinophils

In an earlier communication, it was shown that changes in the eosinophil count are best compared on the basis of the percentage change from the initial level (10) rather than the absolute difference in the cell counts. The same method is employed in this report.

Experiments on rats

The achievement of stable eosinophil levels by anesthesia, adrenalectomy or hypophysectomy. The mere handling of rats appeared to be followed by fluctuations in the eosinophil counts from hour to hour; and, indeed, the procedure of blood taking was invariably followed by a disappearance of circulating eosinophils within four hours. The magnitude of the fall was 78 per cent of the control level. Under light nembutal sedation (0.3 mg./Gm.

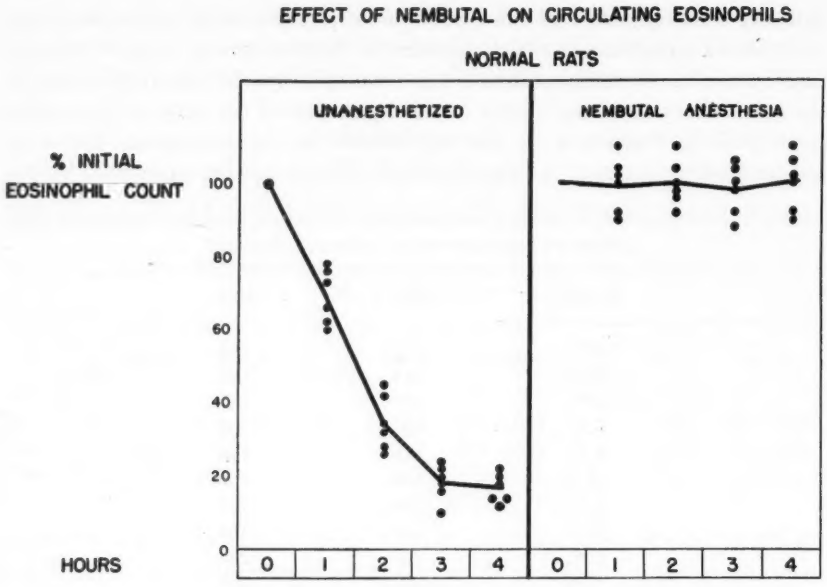

FIGURE 1

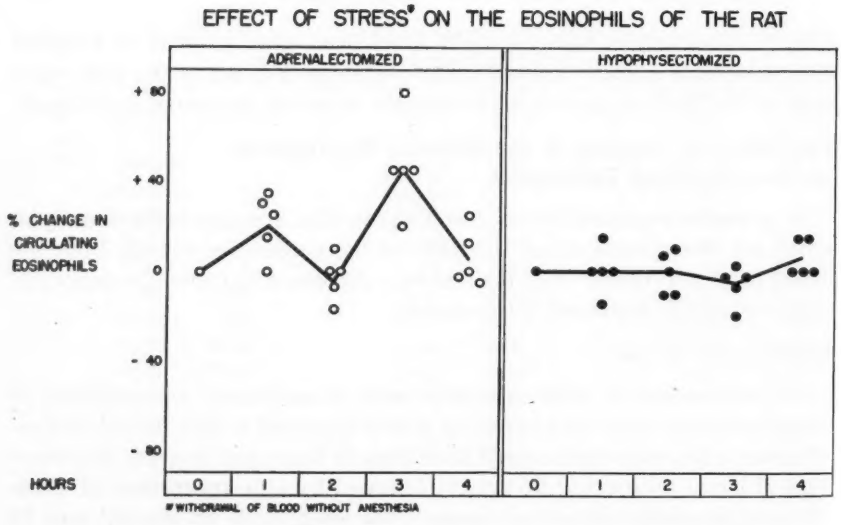

FIGURE 2

TABLE 4. THE RESPONSE OF RAT EOSINOPHILS TO STRESS, TO ACTH AND TO EPINEPHRINE
(Animals studied under nembutal anesthesia)

Stress (blood withdrawal)			ACTH (1.5 mg. intraperitoneally)			Epinephrine (0.1 mg. intraperitoneally)		
Eosinophils per cu. mm.		Per cent change	Eosinophils per cu. mm.		Per cent change	Eosinophils per cu. mm.		Per cent change
0 hrs.	4 hrs.	4 hrs.	0 hrs.	4 hrs.	4 hrs.	0 hrs.	4 hrs.	4 hrs.
Normal								
360	65	−82	380	53	−83	356	107	−70
830	150	−82	500	90	−82	420	84	−80
1,300	280	−84	750	135	−82	1,000	250	−75
760	167	−78	1,000	200	−80	850	340	−60
750	225	−70	1,000	220	−78	900	461	−60
3,090	804	−74	1,500	360	−76	1,200	624	−48
	Mean	−78%		Mean	−80%		Mean	−66%
Adrenalectomized								
940	940	0	850	810	−5	900	1720	+80
850	850	0	790	740	−6	700	1050	+50
1,220	1,221	0	950	949	0	620	745	+20
1,610	1,915	+10	1,200	1,224	+2	1,500	2,250	+50
1,000	1,200	+20	1,240	1,289	+4	1,000	1,500	+50
1,100	1,375	+25	1,400	1,456	+4	1,200	1,800	+50
	Mean	+9%		Mean	0%		Mean	+50%
Hypophysectomized								
750	749	0	1,900	950	−50	740	738	0
600	602	0	850	442	−52	620	621	0
800	800	0	880	400	−56	850	843	− 1
850	1,020	+20				900	990	+10
920	1,012	+10				880	968	+10
	Mean	+6%		Mean	−53%		Mean	+4%
Demedullated								
			200	36	−82			
			580	128	−78			
			490	117	−76			
			790	315	−60			
			880	530	−40			
				Mean	−67%			

of body weight), intraperitoneally administered, it was noted that the eosinophil level became stable within two hours and did not fall in a four-hour period of observation in spite of repeated blood withdrawal (Fig. 1).

The handling of unanesthetized but adrenalectomized and hypophysectomized rats was not accompanied by any significant fall in circulating eosinophils. In the 6 hypophysectomized animals, the eosinophils remained stable (Fig. 2 and Table 4); whereas eosinophil counts in the adrenalectomized animals showed definite swings during the four-hour period of observation, but the strikingly sharp curve of descent noted in the normal unanesthetized animals did not occur. This finding, as well as an occasional marked fall after what appeared to have been a complete adrenalectomy, suggests the presence of small adrenal cortical remnants capable of activation by stress. Postoperative desoxycorticosterone administration tends to suppress the hyperplasia of cortical remnants.

It would appear that the marked fall in circulating eosinophils following the stress of handling and blood withdrawal in rats is largely dependent upon adequate sensory preception and the presence of intact pituitary-adrenal function.

Experiments on dogs

The dependence of the eosinopenia of stress on the presence of an intact pituitary-adrenal system. Following hypophysectomy in rats rapid atrophy of the adrenal cortex occurs leading to a 50 per cent reduction in size by the sixth day (21). This change in the dog is somewhat less rapid, and for this reason the dog was used for the study of the role of the anterior pituitary in the eosinophil response to stress. After simple hypophysectomy, in which fragments of pars tuberalis are left behind, the animal continues to show an eosinopenic response to ACTH for two to four weeks. During this time the response gradully decreases and finally disappears. After total hypophysectomy, the eosinopenic response to ACTH disappears within seven days. If the adrenal cortices of these dogs are maintained with small doses of ACTH, they continue to show normal eosinopenic responses to test doses of ACTH.

Six normal dogs were subjected to major surgery under pentothal-induced ether anesthesia. This type of anesthesia did not produce a significant eosinopenia by itself. The operative procedures included nephrectomy, unilateral adrenalectomy, hypophysectomy and cardiotomy. Four to six hours following the beginning of the operation, circulating eosinophils had fallen 82 per cent to 97 per cent from the original levels, with a mean fall of 91 per cent (Fig. 3). In contrast, 2 bilaterally adrenalectomized dogs that did not respond to ACTH showed no significant fall in eosinophils when subjected to unilateral nephrectomy (Fig. 3).

The response to stress was then studied in 2 dogs with simply hypophysectomy, and 1 dog with total hypophysectomy, that were given maintenance doses of ACTH. Seven to fifteen days after hypophysectomy, the

animals were subjected to unilateral nephrectomy. The response to operation in all 3 animals was a *rise* in eosinophils averaging 52 per cent, in contrast to the 91 per cent fall in the normal group (Fig. 3). That the absence of the pituitary and not a deficiency in adrenal cortical function alone accounted for this effect was shown by the eosinopenic response to ACTH in these dogs.

It would appear that a major stress depresses the number of circulating

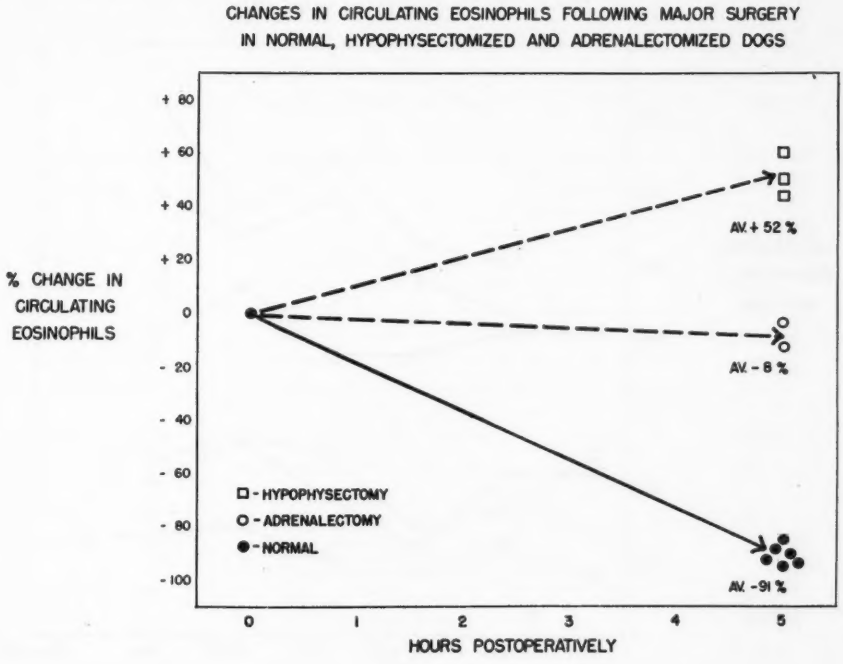

FIGURE 3

eosinophils through the intermediary action of both the anterior pituitary and the adrenal cortex in the dog.

Experiments on man

Eosinopenia following severe stress as a reflection of pituitary-adrenal integrity. The fasting level of circulating eosinophils is subject to considerable variation depending upon the severity of the physical or emotional stress to which the individual is exposed. Under constant conditions such as in a hospital, the levels show little change. Even the diurnal variations observed in 6 young males, subject to the exigencies of general hospital

work, did not exceed 40 per cent of the initial levels. The curves were characterized by a gradual afternoon rise in eosinophils (Fig. 4).

Eosinopenia follows the stress of acute pain, chronic starvation and agony of many types in subjects with normal adrenocortical function; whereas, under comparable circumstances, Addisonians fail to show a significant fall in their eosinophils. In Cushing's syndrome a marked eosino-

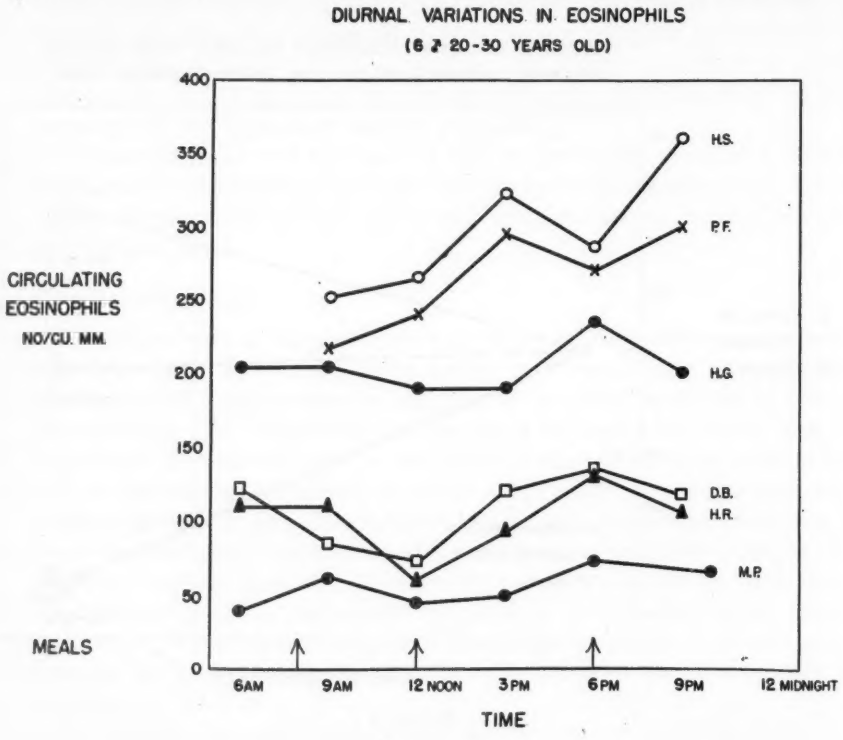

FIGURE 4

penia is noted in the absence of stress. This is compatible with the maintained state of adrenal cortical overactivity (Table 5).

Major surgery also acts as a depressor of circulating eosinophils in patients with normal adrenal cortical function. In a group of 12 patients studied five to fourteen hours after a major operation, a 95 to 100 per cent fall took place. The return toward normal began after two days and the level often exceeded the original in a week. One patient who subsequently died of intractable shock showed a relatively high level of eosinophils six hours after operation. Autopsy revealed caseous destruction of both adrenals, which had been entirely unsuspected preoperatively.

These observations demonstrate that the development of an eosinopenia characterizes the response of normal man to stress and that the pituitary-adrenocortical system is involved in this reaction.

It is obvious from the effects of stress on the eosinophil in the rat, the dog and man that in order to compare the action of various agents on the

TABLE 5. RESPONSE OF EOSINOPHILS TO STRESS IN MAN

Patient		Diagnosis	Eosinophils per cu. mm.
P.M.	(M)	Acute coronary occlusion	16
L.C.	(M)	Acute coronary occlusion (1½ hrs. post.)	90
		(5½ hrs. post.)	9
		(12 hrs. post.)	4
A.Y.	(F)	Anorexia nervosa (terminal)	0
J.L.	(M)	Hepatic coma and cachexia	4
H.M.	(M)	Hepatic coma and hemochromatosis	0
F.B.	(F)	Whipple's disease (terminal)	36
I.H.	(F)	Acute gastro-intestinal upset	0
M.R.	(F)	Pyrogenic reaction (102° F.)	5
		Addison's disease	
E.C.	(M)	Suppurative parotitis	250
		Hyperpyrexia (104° F.)	184
J.P.	(M)	Acute upper respiratory infection	100
J.C.	(F)	Bronchopneumonia	96
		Cushing's syndrome without stress	
G.W.	(F)	Cushing's syndrome	46
M.G.	(F)	Cushing's syndrome	26
A.R.	(F)	Cushing's syndrome	23
G.W.	(M)	Cushing's syndrome	20
L.D.	(F)	Cushing's syndrome	5

eosinophil, either a subject devoid of adrenal cortical response must be used or else the imposition of any type of stress must be studiously avoided. It would appear that because of its sensitivity as an indicator, the eosinophil may react to small changes in adrenal cortical secretion unaccompanied by any other measurable physiologic or biochemical effect.

The Effect of Adrenal Cortical Hormone on the Circulating Eosinophils

Adrenalectomized animals and patients with Addison's disease were chosen for study. This eliminated the variations imposed by the natural alarm response.

Experiments on rats

Four anesthetized adrenalectomized rats were injected intraperitoneally with normal saline and their eosinophils were followed over a four-hour period (Fig. 5). No significant fall in eosinophils occurred in this group, whereas an 82 per cent fall occurred in 6 adrenalectomized animals injected with 3 cc. of aqueous adrenal cortical extract A (Eschatin, Parke, Davis & Co.) within three and one-half hours (Fig. 5). When 1 cc. of extract was used, only a 42 per cent fall was observed. A 66 per cent fall in eosinophils was noted in 3 rats given 3 mg. of Compound F (17-OH cor-

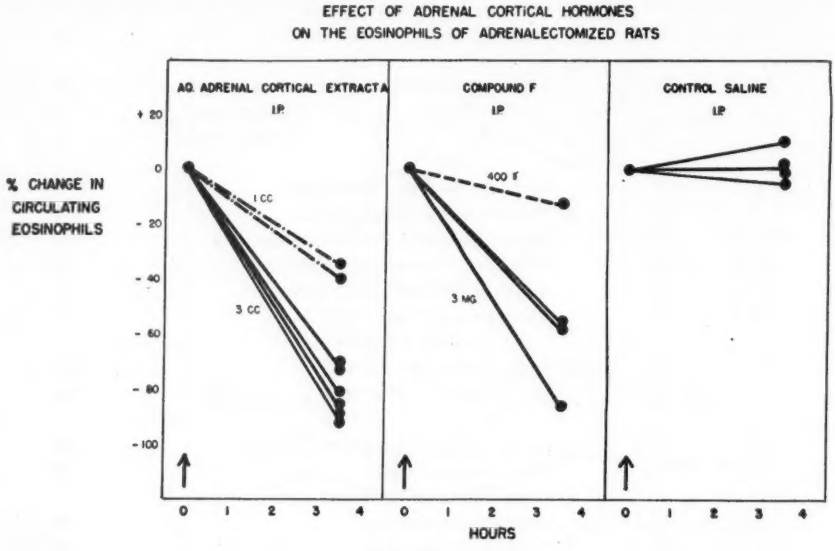

FIGURE 5

ticosterone) intraperitoneally in saline, although no significant change was noted with 400 gammas of F (Fig. 5).

Experiments on dogs

Three adrenalectomized dogs were given 15 mg. of Compound E acetate intravenously in 150 cc. of saline over a period of one hour as previously described. A mean fall of 59 per cent in circulating eosinophils, and a 60 per cent fall in circulating lymphocytes with a moderate increase in the polymorphonuclear leukocytes was observed within four hours. Similarly administered, 25 cc. of Wilson's Adrenal Cortex Extract led to a 30 per cent fall in eosinophils. Upon the addition of 0.3 mg. of epinephrine to 25 cc. of extract, a 27 per cent fall was obtained. Thus no "potentiation" occurred.

Experiments on man

All patients studied were Addisonians who had failed to show a response to ACTH. Aqueous adrenal cortical extract A (Eschatin, Parke, Davis & Co.) was administered in 50 cc. doses intravenously in 150 cc. of saline over the course of three quarters of an hour. A mean eosinophil fall of 56 per cent was produced in 5 patients within four hours (Table 6).

TABLE 6. PERCENTILE FALL IN CIRCULATING EOSINOPHILS FOLLOWING INTRAVENOUS ADMINISTRATION OF ADRENAL CORTICAL EXTRACT* TO PATIENTS WITH ADRENAL INSUFFICIENCY

Patient	Eosinophils, % fall
R.R.	84
J.P.	79
	46
C.S.	51
	53
	58
C.N.	57
M.H.	31
Mean percentile fall:	59

* 50 cc. of Eschatin (Parke, Davis & Co.) in 100 cc. of saline administered over a one-hour period intravenously.

Seven Addisonians given Compound E Acetate intravenously (20–50 mg.) over a 30 to 60-minute period showed a mean four-hour eosinophil fall of 52 per cent (range: 18 per cent to 73 per cent).

The typical effect of various adrenal steroid preparations on the circulating eosinophils in a representative patient with Addison's disease is shown in Figure 6.

These experiments extend the previous findings that 20 mg. of Compound F (17 hydroxycorticosterone) administered intramuscularly leads to an average fall of 62 per cent in eosinophils in patients with Addison's disease, whereas 50 mg. of desoxycorticosterone glucoside fails to depress eosinophils significantly (10) (Fig. 6).

From these experiments in rat, dog and man, it may be concluded that certain adrenal cortical extracts or steroids of the 11–17 oxy-type show a specific effect upon the level of circulating eosinophils and lymphocytes and

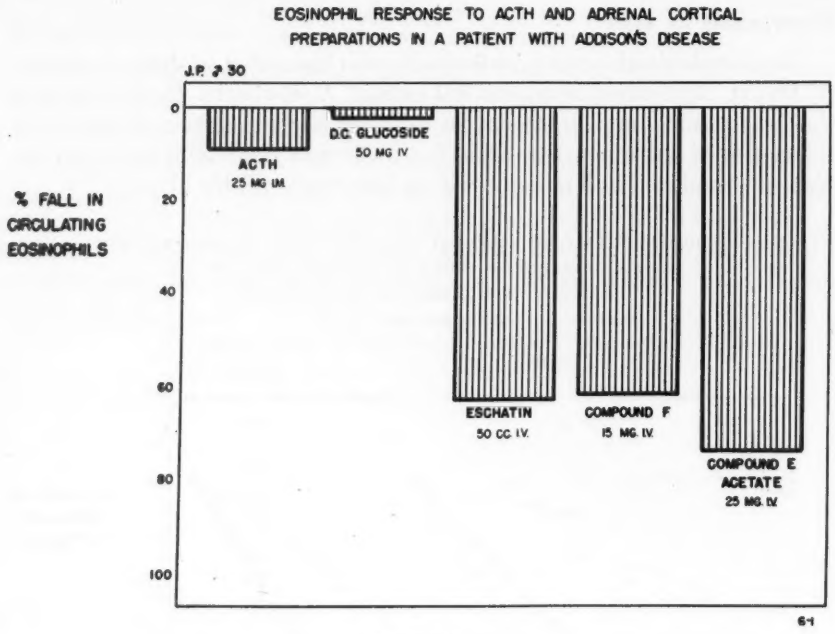

FIGURE 6

(DC glucoside refers to desoxycorticosterone glucoside.)

appear to be largely responsible for the eosinopenia following adrenal cortical stimulation.

The Effect of Adrenocorticotropic Hormone on the Circulating Eosinophils

ACTH was used as a direct stimulant of the adrenal cortex and the changes in circulating eosinophils were followed.

Experiments on rats

ACTH was administered intraperitoneally in a dosage of 1.5 mg. to groups of 6 rats. Normal and demedullated rats required anesthesia in order to stabilize their eosinophil levels, whereas the adrenalectomized and hypophysectomized groups could be studied without anesthesia. However, all animals were studied under light sedation in order to maintain constancy.

In normal animals, an 80 per cent eosinophil fall occurred within four hours. There was no eosinophil fall in the group of adrenalectomized animals in which the presence of accessory cortical tissue was excluded (Fig. 7 and Table 4). An average fall of 67 per cent was observed in the demedul-

lated animals. The extensive scattering of the results in the demedullated animals is noteworthy. This is presumably related to incomplete regeneration of the adrenal cortex, as late as four weeks following the demedullation procedure (Fig. 7 and Table 4).

These findings suggest that failure of adrenalectomized animals to show an eosinopenia in response to ACTH is dependent not on the removal of the adrenal medulla, but on the absence of the cortex. Direct stimulation

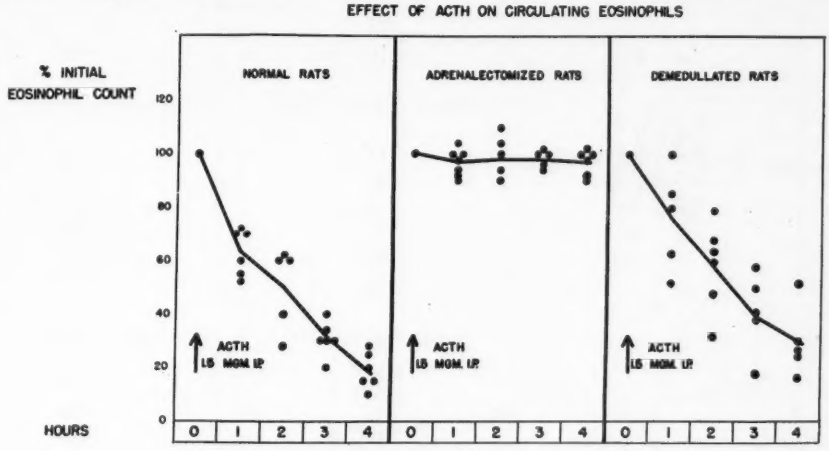

FIGURE 7

of the adrenal cortex of the rat by ACTH appears to produce an eosinopenia irrespective of the presence of the adrenal medulla.

Experiments on dogs

An equivalent of 15 mg. of the Armour standard ACTH was administered (intramuscularly) to 6 normal and 6 adrenalectomized dogs. Cell counts were determined at zero time and four hours after the injection. Eosinophils fell 67 per cent in the normal animals, whereas a rise of 1 per cent occurred in the adrenalectomized group (Table 7). In 1 normal animal studied, a 60 per cent fall in lymphocytes and a 66 per cent rise in total white count were also observed, whereas none of these changes occurred in the adrenalectomized animal (Table 8).

In dogs, ACTH appears to induce hematologic changes identical with those observed following the administration of 11-17 oxysteroids.

Experiments on man

Previous work from this laboratory has shown that a 78 per cent fall in eosinophils follows the intramuscular administration of 25 mg. of ACTH to normal subjects. Patients with Addison's disease show only a 5 per cent

TABLE 7. EOSINOPHIL RESPONSE TO ACTH IN RAT, DOG AND MAN

ACTH dose	Species	*Normal*	*Adrenalectomized*	*Hypophysectomized*
1.5 mg.	*Rat*	−86	− 5	−50
		−82	− 6	
		−82	0	−52
		−80	+ 2	
		−78	+ 4	
		−76	+ 4	−56
		Mean −80%	Mean 0%	Mean −53%*
15 mg.	*Dog*	−51	− 1	−48
		−60	0	−51
		−62	0	−53
		−68	0	−70
		−75	+ 3	
		−84	+ 4	
		Mean −67%	Mean + 1%	Mean −56%
25 mg.	*Man*†	−70	− 9	0
		−90	+ 7	−25
		−89	+ 1	−27
		−82	0	−37
		−55	− 2	−38
		−66	−11	−45
		Mean −74%	Mean + 1%	Mean −29%

* Animals were given 2 mg. of ACTH every eight hours intramuscularly postoperatively for forty-eight hours and the final test was performed eight hours after the last dose.

† Patients with Addison's disease and pituitary insufficiency are represented.

TABLE 8. TYPICAL HEMATOLOGIC RESPONSE TO ACTH IN DOGS
(15 mg. intramuscularly)

	Normal dog			*Adrenalectomized dog*		
	0 hrs.	4 hrs.	Per cent	0 hrs.	4 hrs.	Per cent
Eosinophils	2,550	525	−79	2,660	2,800	+5
White count	15,400	23,350	+66	17,500	17,550	0
Lymphocytes	3,850	1,524	−60	6,150	6,050	−1

fall (16, 22). The expanded series of cases of 43 Addisonians and 20 normal subjects show an average 76 per cent fall for normals and an 8 per cent fall for Addisonians (Fig. 8).

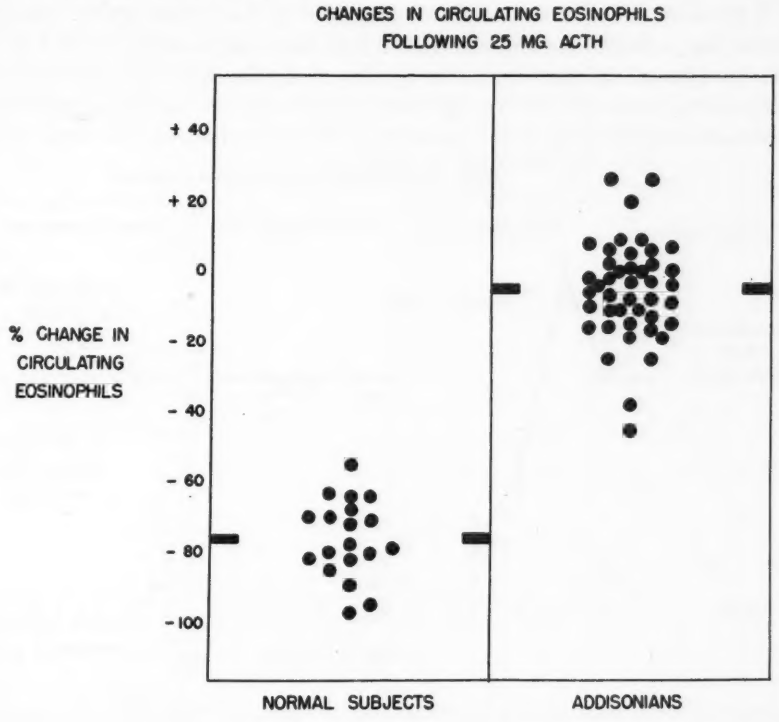

FIGURE 8

From these observations on the effect of ACTH upon the eosinophils of rats, dogs and man, it appears that eosinopenia results from the release of adrenal cortical hormones, and not from a direct effect of the ACTH on the circulating eosinophil.

The Effect of Epinephrine on the Circulating Eosinophils

Having demonstrated that the depression of circulating eosinophils may be used as an indicator of increased adrenal cortical secretion, it seemed of interest to study the effect of epinephrine upon these cells.

Experiments on rats

Normal, splenectomized, adrenalectomized, and hypophysectomized rats (ten days postoperatively) in groups of 6 animals each, were given

0.1 mg. of aqueous epinephrine hydrochloride (1:1000) intraperitoneally, and hourly eosinophil counts were performed. The eosinophil counts were stabilized by nembutal anesthesia as previously described.

A biphasic curve of response was observed in the normal group, characterized by a rise in eosinophils in the first hour and a 66 per cent fall by the third hour. Splenectomized rats showed similar behavior. Neither the adrenalectomized nor the hypophysectomized animals showed a significant fall in eosinophils (Fig. 9). The absence of functional adrenal cortical tissue

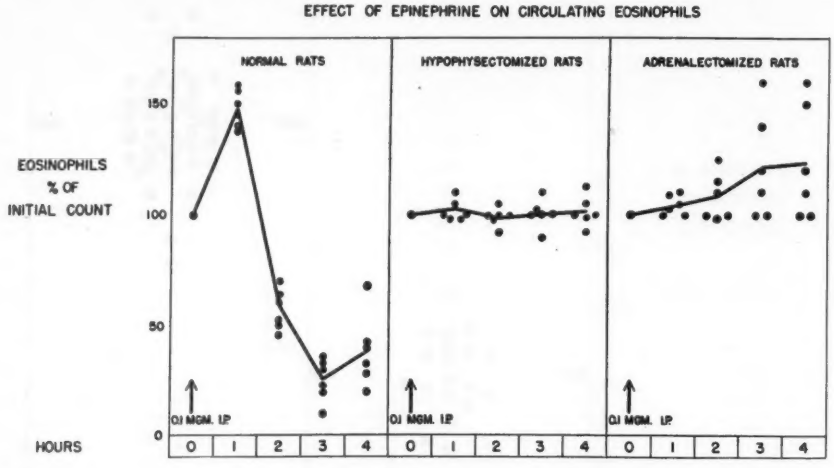

FIGURE 9

appeared to be the limiting factor in the two groups which did not respond. In order to study the epinephrine response in the absence of the pituitary but in the presence of functional adrenal cortex, 3 hypophysectomized rats were maintained on 10 mg. of ACTH daily in four divided doses starting six hours after the operation. The presence of functional adrenal tissue appeared certain, in that a 53 per cent fall in eosinophils was observed following ACTH administration. However, epinephrine administered eight hours after the last dose of ACTH still failed to evoke an eosinopenia in these animals (Table 7).

These experiments indicate that the eosinopenia following epinephrine is dependent on an increase in adrenal cortical activity. They also support the view that epinephrine stimulates the adrenal cortical secretions through an activation of pituitary ACTH.

Experiments on dogs

Six normal dogs were infused with 0.2 mg. of epinephrine hydrochloride (1:1000) in 150 cc. of saline over a one-hour period. In this group, an

average fall in eosinophils of 50 per cent occurred within four hours. In subsequent studies, 0.3 to 1.0 cc. of aqueous epinephrine hydrochloride (1:1000) was administered subcutaneously with approximately the same effects.

In 6 adrenalectomized animals the eosinophils rose at the end of the four-hour period (Fig. 10). No fall was observed even with doses up to 1 mg. of epinephrine.

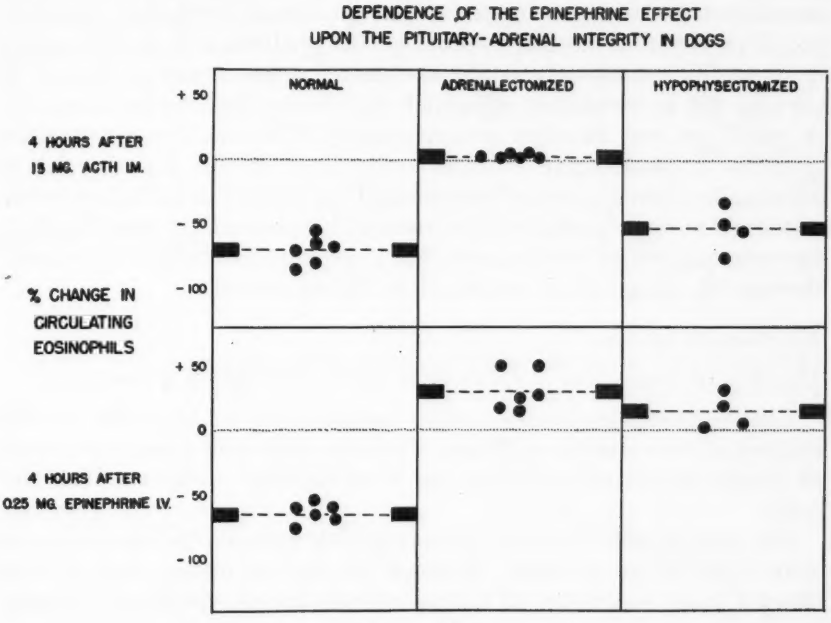

FIGURE 10

(Heavy bars indicate the mean change in the eosinophil level in the group.)

Three dogs were subjected to total hypophysectomy and 1 animal had only the anterior lobe of the pituitary removed. The functional integrity of the adrenal cortex was shown to be fully maintained up to twelve days postoperatively by obtaining a mean fall in circulating eosinophils of 50 per cent following the intramuscular administration of 15 mg. of ACTH (Fig. 10 and Table 7). None of these animals showed any depression of eosinophils when given epinephrine. All showed a rise ranging from 7 to 32 per cent over the initial levels (Fig. 10).

In contrast, 2 animals subjected to sectioning of the pituitary stalk, (severing any direct neural connections with the hypothalamus) showed a normal fall in eosinophils in response to epinephrine and ACTH.

These data suggest that epinephrine leads to an eosinopenia through stimulation of the anterior pituitary ACTH production. This stimulation appears to be humoral in character and independent of nervous connections of the pituitary with the hypothalamus. It should be noted, however, that this does not necessarily represent a direct action of epinephrine on the anterior pituitary itself.

Is epinephrine the obligatory intermediary in the activation of the anterior pituitary ACTH mechanism in stress? The normal response of demedullated rats to stress suggested that the adrenal medullary contribution of epinephrine was not essential for the production of an eosinopenia in stress. Two completely sympathectomized dogs showed an 83 and 86 per cent fall in circulating eosinophils four hours after the injection of 2 cc. of 37 per cent formalin intramuscularly. This was not accompanied by a rise in blood sugar. Normal control dogs showed a similar fall in eosinophils, whereas a hypophysectomized dog showed no fall. There would appear to be some mechanism for eosinophil depression through the pituitary-adrenal system which represents a response to stress not mediated through the sympathetic or adrenal medullary system.

Experiments on man

The effect of single doses of epinephrine over a short period of time.

Epinephrine hydrochloride in doses ranging from 0.2 to 1.5 mg. was administered intravenously in 200 cc. of normal saline over a one-hour period. In another series, from 0.2 to 0.5 mg. of epinephrine was given subcutaneously.

The *clinical effects* usually associated with epinephrine administration were observed on occasion. Whereas the smaller dosage showed little effect, 1.5 mg. invariably led to classic symptoms of sympathetic stimulation which subsided soon after the end of the infusion.

The *general hematologic changes* following epinephrine infusion were characterized by biphasic leukocytic curves as shown for a group of 6 normal subjects in Figure 11. The behavior of the various leukocytes differed significantly, though all showed an initial rise in the first hour. The lymphocytes showed a transitory fall (30 per cent) after two hours and returned to their previous level in four hours. At this time the eosinophils were maximally depressed (68 per cent). Along with these changes, the hematocrit never varied more than 1 to 2 per cent. The biphasic curve was seen best after the administration of 1.5 mg. of epinephrine and rarely with 0.3 mg. The fall in eosinophils was found to occur even in the absence of the initial rise. The previous administration of ACTH to a young male abolished the initial rise in eosinophils following 1.5 mg. of epinephrine intravenously, without in any way changing the four-hour fall (Fig. 12). The lymphocytes, however, showed the identical biphasic curve in each

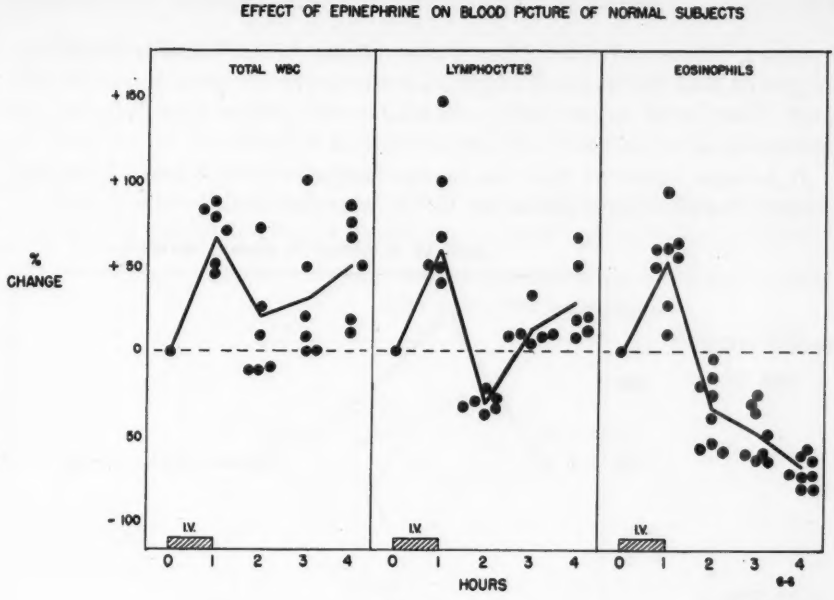

FIGURE 11

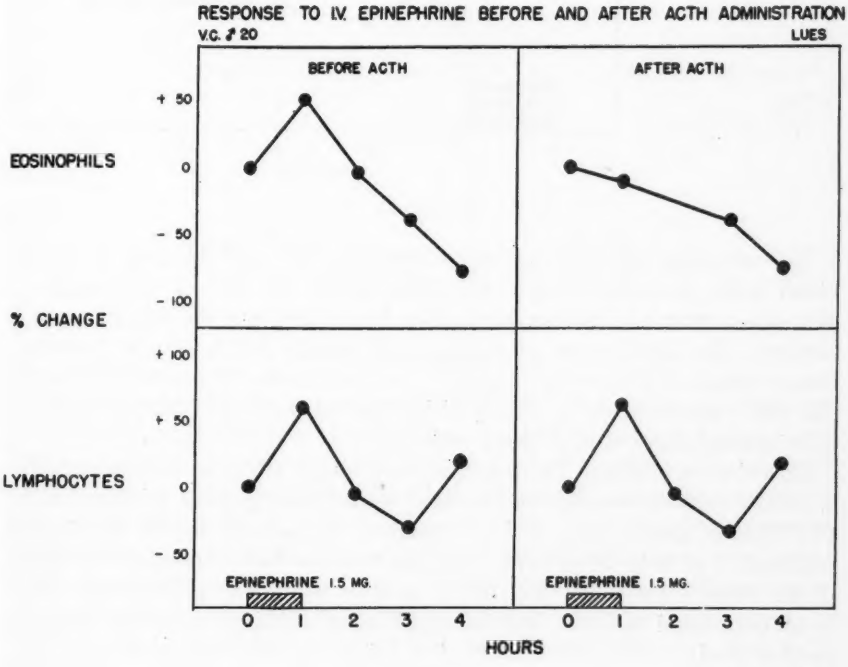

FIGURE 12

instance, before and sixteen hours after 25 mg. of ACTH. These findings suggested that the reservoir of lymphocytes is greater than that of eosinophils. This could conceivably account for the greater sensitivity of the eosinophil as an indicator of adrenal cortical secretion.

It became apparent that the hematologic effects four hours after the administration of epinephrine or ACTH were identical.

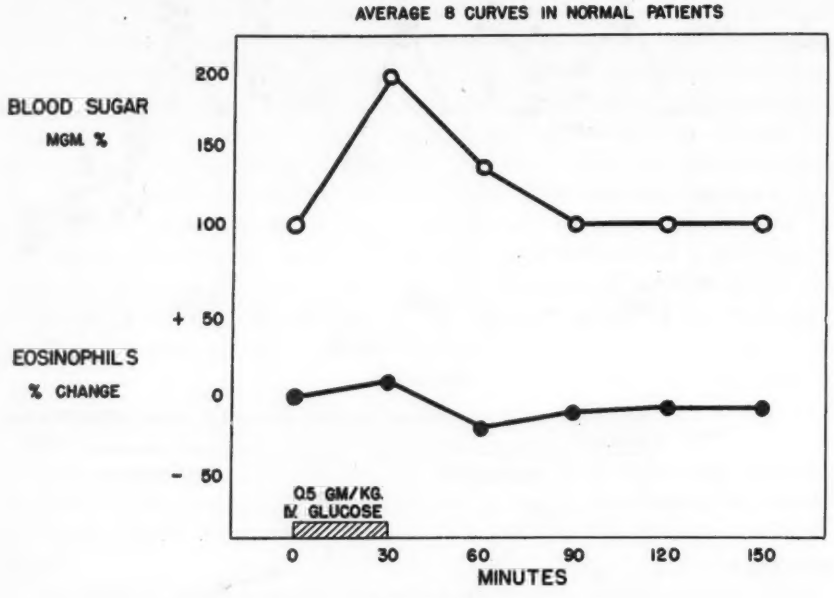

FIGURE 13

The *metabolic effects* following epinephrine were not striking. A rise in blood sugar occurred, which was maximal at the end of the infusion. Whereas a return to fasting levels after four hours was the rule in normal subjects, the blood sugar in Addisonians usually fell below, and in diabetics remained above the respective control levels. This adrenocortical-like effect appeared to be clearly differentiated from the early, glycogenolytic epinephrine-induced blood sugar rise.

In order to evaluate the possible eosinopenic effect of hyperglycemia, 8 normal subjects were given standard intravenous glucose tolerance tests (0.5 Gm. of glucose/Kg. of body weight). A maximal fall in circulating eosinophils of only 20 per cent was reached one half hour after the peak of the blood sugar curve (Fig. 13). A greater and prolonged fall was noted in an occasional diabetic, but the significance of this observation requires further study.

The induction of a transitory hyperglycemia (180 mg. per cent) did not produce an eosinopenia in an adrenalectomized dog and fasting eosinophil levels were found to be high in a diabetic Addisonian at blood sugar levels of from 300 to 400 mg. per cent. Such findings make a direct eosinopenic action of hyperglycemia unlikely.

That this small but consistent fall in eosinophils following intravenous glucose administration might represent a minimal adrenocortical response,

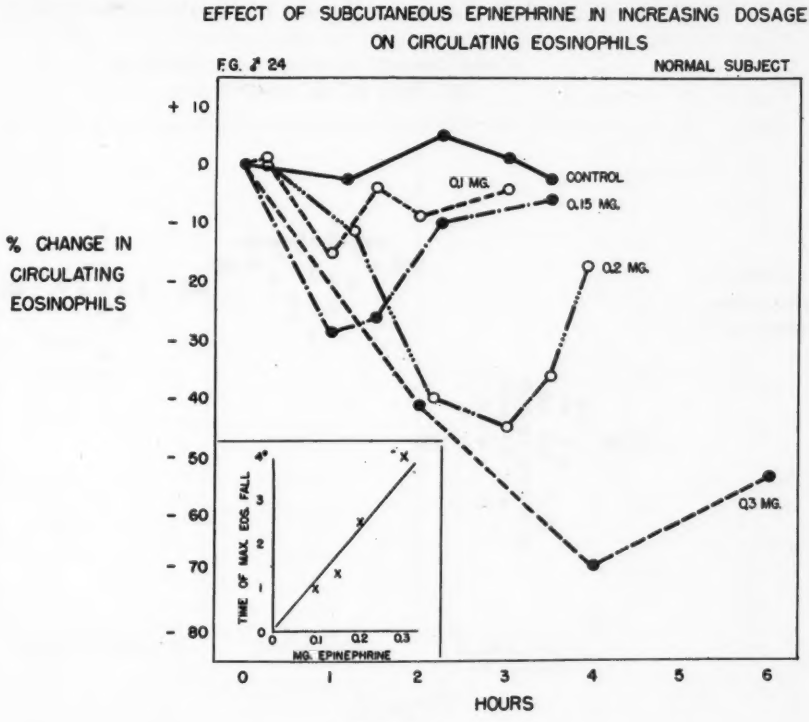

FIGURE 14

is suggested by the similar eosinophil response following the subcutaneous injection of small amounts of epinephrine. As may be gathered from Figure 14, not only is the depression of circulating eosinophils smaller with the small doses of epinephrine, but the time of maximal depression appears to be a function of the dosage employed. Thus, minimal adrenal cortical stimulation appears to lead to an early and minor fall in circulating eosinophils, not unlike that seen in the intravenous glucose tolerance curve.

In contrast to the ACTH-induced rise in the urinary uric acid-creatinine ratio, epinephrine failed to induce any such change (16).

The *eosinopenic effect of epinephrine* was the most constant finding noted. The administration of *0.2 mg. of epinephrine*, intravenously, in 200 cc. of saline over a one-hour period, led to a mean fall in eosinophils of 60 per cent (range 44 to 91 per cent) in 35 subjects known to have normal pituitary adrenal cortical reserve. The standard deviation for the group was 4 per cent. Thirty-one cases of classic Addison's disease were found to show a mean fall in circulating eosinophils of only 7 per cent (range +3 to −55 per cent) and the standard deviation was 5 per cent. Twelve cases of pitui-

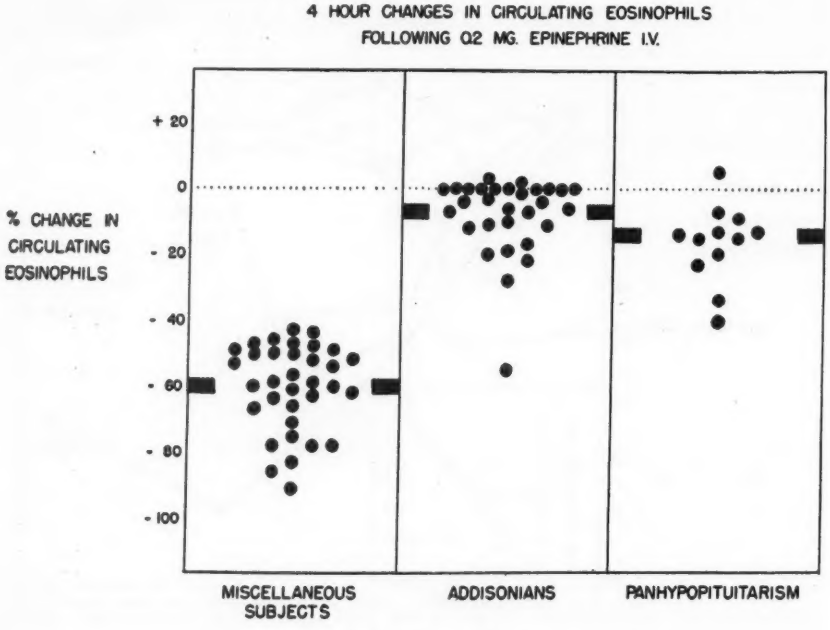

FIGURE 15

(Heavy bars indicate the mean change in the eosinophil level in the group.)

tary insufficiency showed a mean fall in circulating eosinophils of 18 per cent (range +5 to −40 per cent). Whenever the response approached the normal, there was minimal clinical evidence of adrenal insufficiency (Fig. 15).

It is apparent that there is a significant difference in the eosinophil response between subjects with normal adrenal cortical reserve and those with a limited or absent reserve, when doses of epinephrine *not exceeding 0.3 mg.* are used in the standard intravenous fashion.

TABLE 9. EFFECT OF SUBCUTANEOUS EPINEPHRINE (0.3 MG.) ON CIRCULATING EOSINOPHILS IN MISCELLANEOUS SUBJECTS AND IN PATIENTS WITH ADDISON'S DISEASE

Patient	Diagnosis	Eosinophils per cu. mm.		Percentage change
		Initial	4 hr. level	
A.S.	Lues	159	72	−55
C.M.	Healed fracture	173	75	−57
J.P.	Lues	172	87	−49
N.R.	Normal	128	56	−56
R.R.	Normal	123	33	−73
E.L.	Normal	67	27	−60
E.W.	Normal	262	123	−53
F.W.	Normal	97	42	−56
R.M.	Normal	142	42	−66
N.A.	Normal	117	23	−80
C.M.	Normal	136	44	−68
F.G.	Normal	178	50	−72
Mean		146	56	−62
Range		(67–262)	(23–123)	(49–80)
F.H.	Addison's disease	114	137	+20
V.A.	Addison's disease	283	262	− 7
M.H.	Addison's disease	125	112	−10
D.D.	Addison's disease	152	127	−16
H.McC.	Addison's disease	154	127	−18
M.N.	Addison's disease	177	142	−20
M.R.	Addison's disease	103	81	−21
M.J.	Addison's disease	131	98	−25
J.R.	Addison's disease	100	73	−27
E.D.	Addison's disease	441	317	−28
W.D.	Addison's disease	86	48	−44
Mean		162	133	−21%
Range		(86–441)	(48–317)	(+20 to −44)

The same dose subcutaneously yields results approaching those of the intravenous route (Table 9). The bulk of the work was carried out by the intravenous test, chiefly because of better dosage control by this route of administration. However, as a practical test, the subcutaneous route of administration is very satisfactory.

The administration of as much as *1.5 mg. of epinephrine*, intravenously in 200 cc. of saline over a one-hour period, led to a significant depression in

eosinophils, not only in normal subjects, but in Addisonians. Eight patients with Addison's disease and 10 normal subjects were studied. Although the Addisonians had shown only a small response to 0.2 mg. of epinephrine or 25 mg. of ACTH, a mean fall of 48 per cent was observed with 1.5 mg. of epinephrine. The normal subjects showed a 68 per cent fall (Fig. 15). The administration of 1.5 mg. of epinephrine intravenously failed to depress the eosinophils significantly in 4 cases of severe pituitary insufficiency (Fig. 16), although ACTH did show some effect in 3 of the 4 cases.

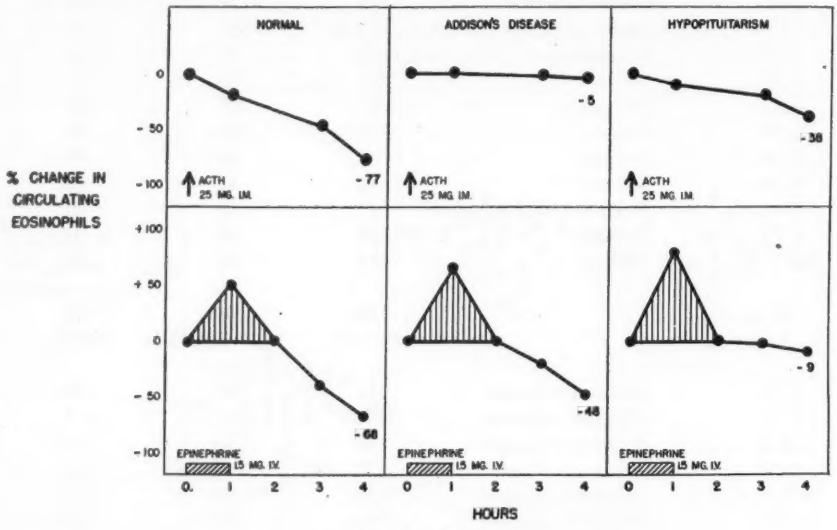

FIGURE 16.

(The curves represent the averages of 10 normal subjects, 8 patients with Addison's disease and 4 patients with panhypopituitarism, respectively.)

The somewhat unexpected response of patients with Addison's disease to the large dose of epinephrine is in sharp contrast to the minimal response with the small dose. The absence of any response to epinephrine in adrenalectomized dogs and certain cases with panhypopituitarism supports the assumption that patients with Addison's disease still have some residual adrenal cortical function and that the larger dose of epinephrine may be a more effective pituitary ACTH stimulant than the 0.2 mg. dose. This was further supported by an experiment on one patient (E.C.) with Addison's disease. No fall in eosinophils followed 0.2 mg. of epinephrine; a 5 per cent increase occurred after 25 mg. of ACTH; but a 50 per cent fall was found after 1.5 mg. of epinephrine. In this patient 100 mg. of ACTH administered intramuscularly produced a 16 per cent fall in eosinophils within four hours. This response suggested the presence of some functional adrenal cortical

tissue which did not, however, appear capable of exerting the same effect on the eosinophils when stimulated by 100 mg. of exogenous ACTH, as following the endogenous ACTH secreted in response to 1.5 mg. of epinephrine.

The eosinopenic action of cortical extract may be enhanced by epinephrine-induced steroid secretion in patients with Addison's disease, as suggested by the following experiments:

Three aqueous adrenal cortical extracts (A, B, C) were administered to Addisonians who had previously shown no eosinopenia following either

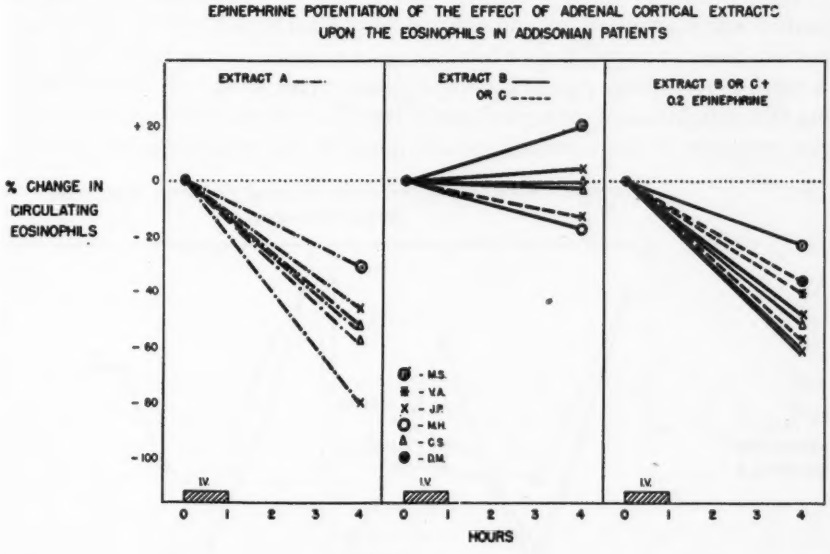

FIGURE 17

0.2 mg. of epinephrine or 25 mg. of ACTH. Fifty cc. of extract in 100 cc. of normal saline was given intravenously over a one-hour period. Extract A produced a definite fall in circulating eosinophils within four hours, while B and C produced no significant change in the eosinophils. It was noted that extract A, unlike B and C, led to physiologic changes suggesting that small quantities of epinephrine-like substances might be present. Following up this observation, extracts B and C, combined with 0.2 mg. of epinephrine, were administered as previously described. In all cases a fall in eosinophils occurred (Fig. 17).

In 2 additional Addisonians, evidence of this action was obtained, in that 20 mg. of Compound E acetate combined with 0.2 of epinephrine produced a somewhat greater fall in eosinophils and lymphocytes than that observed

using E acetate and epinephrine separately. It would appear that quantities of eosinophil-depressing adrenal steroids incapable alone of producing a maximal fall in eosinophils, may be potentiated by the secretion of subliminal quantities of corticoids under epinephrine-induced ACTH stimulation. No such synergism was found in an adrenalectomized dog, demonstrating the dependence of this action on the presence of some adrenal cortical tissue.

The effects of repeated doses of epinephrine.

The optimal frequency for the production of eosinopenia by the repeated administration of epinephrine was established by the use of repeated doses at varying time intervals in a normal, young male (Fig. 18). A relative refractory period for the adrenal cortex within six hours following the initial dose of epinephrine was suggested by the absence of a maximal response of the circulating eosinophils to the administration of sub-

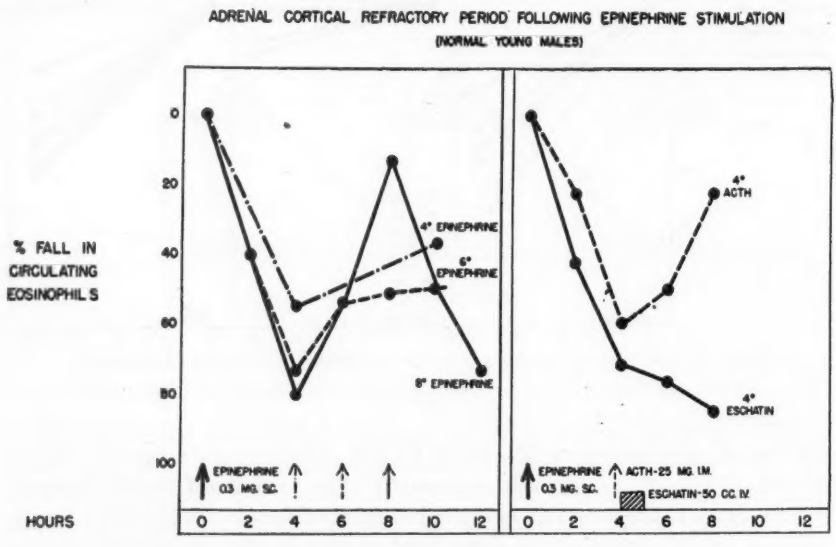

FIGURE 18

cutaneous epinephrine and ACTH. Eschatin at this same time led to a significant eosinophil response. This response established the continued sensitivity of the circulating eosinophil (the end point) as an indicator of adrenal cortical function. Another experiment is shown in Figure 19. On the basis of these observations, it would appear that the optimal frequency of administration lies between six and eight hours.

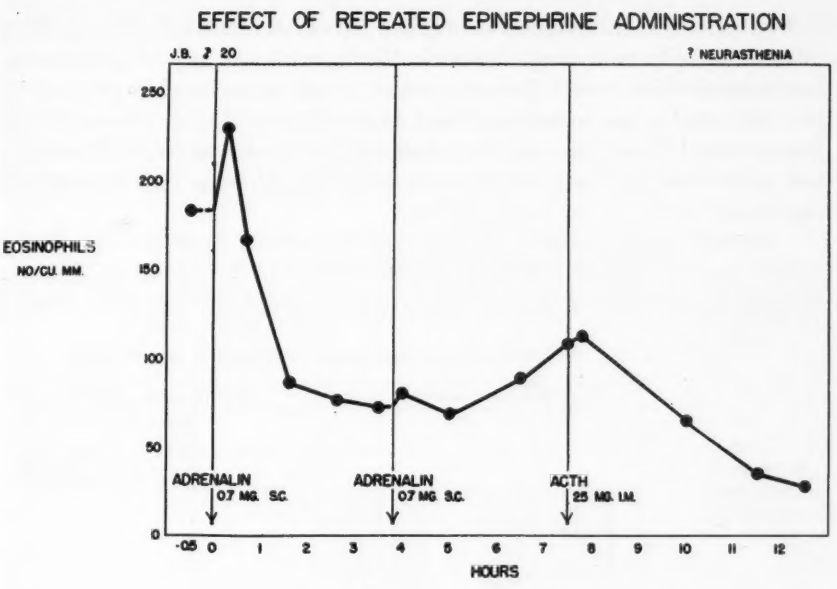

FIGURE 19

A 39-year old female was given injections of 1:1000 epinephrine hydrochloride subcutaneously every six hours for a 48-hour period. The last blood samples were drawn four hours after the last injection. The dosage ranged from 0.5 to 0.8 mg. totalling 6.2 mg. over a 48-hour period, and had to be varied according to the intensity of the physiologic effects induced. Significant changes are summarized in Table 10. A depression of the eosinophils accompanied by a small rise in blood sugar, but no change in the urinary uric acid-creatinine ratio, were noted. The 11-oxysteroid excretion rose to the upper limit of normal.

TABLE 10. EFFECT OF 48-HOUR INTERMITTENT SUBCUTANEOUS EPINEPHRINE ADMINISTRATION

Period of treatment	Dose received, mg.	Eosinophils, No. per cu. mm.	Blood sugar, mg. %	11-Oxysteroids, mg./24 hrs.
Control	0	75	78	0.19
24 Hours	3.2	27	87	
48 Hours	3.0	25	99	0.39

Two normal male subjects were given 1.0 cc. of 1:500 epinephrine in oil intramuscularly every eight hours so that a total of 6 mg. of epinephrine was administered over a 24-hour period. A fall in eosinophils (89 and 60 per cent) and a rise in fasting blood sugar (26 and 32 mg. per cent) four hours after the last injection were observed, but there was no rise in urinary uric acid-creatinine ratio or 17-ketosteroid excretion; in fact a small but significant fall occurred in the latter.

A normal, young medical student who had shown an 86 per cent fall in circulating eosinophils following administration of 25 mg. of ACTH was subjected to a series of 24-hour intravenous epinephrine infusions. During

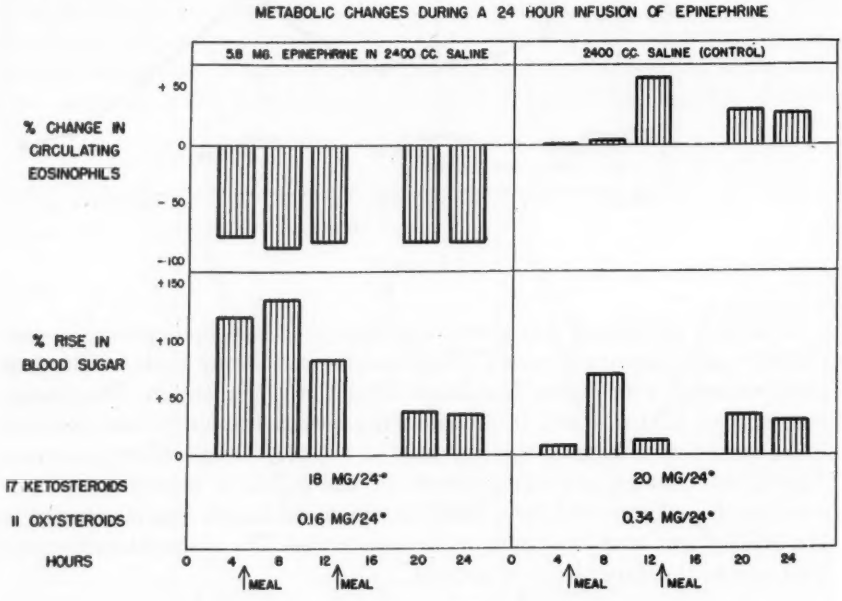

FIGURE 20

the infusion periods, food intake, meal time and urine collections were carefully standardized. An absolute control day was followed by a 24-hour period during which 2400 cc. of saline was given intravenously. On the test day, a 24-hour infusion of 5.8 mg. of epinephrine in 2400 cc. of saline, was administered. A week later a 24-hour infusion of 12 mg. of epinephrine was administered, again followed by a control day.

The eosinophil levels fell, the fasting blood sugars rose, but no change in the urine uric acid-creatinine ratio was observed with either 5.8 or 12 mg. of epinephrine (Fig. 20).

There was no increase in the urinary 11-oxysteroid or 17-ketosteroid excretion. Instead, a definite tendency for the excretion of these steroids to be depressed on the test days, with a return to the control values on the day after the continuous epinephrine infusion was noted (Table 11).

From these studies it must be concluded that stimulation of the pituitary-adrenocortical system by epinephrine over a 48-hour period of time fails

TABLE 11. EFFECT OF CONTINUOUS EPINEPHRINE INFUSION ON THE URINARY EXCRETION OF ADRENAL STEROIDS IN A NORMAL MALE (C.P. 23)

24-Hour period of study	17-Ketosteroids, mg./day	11-Oxysteroids, mg./day
Control	16	0.36
2400 cc. Saline i.v.	20	0.36
2400 cc. Saline and 5.8 mg. epinephrine i.v.	18	0.16
2400 cc. Saline and 12 mg. epinephrine i.v.	10	0.34
Control—one day after 12 mg. of epinephrine	19	0.32

to activate the adrenal cortex of normal adults significantly. With constant administration, and even with repeated separate doses, the activation does not compare in extent to either stress or ACTH, at least with the quantities of epinephrine employed. The eosinophils, as sensitive indicators of adrenal cortical activity, show a definite fall. Yet the amounts of adrenal cortical hormone produced by epinephrine action appear too small to increase the urinary overflow of cortical steroids significantly or to produce marked metabolic changes. It may be that the increased titer of adrenal steroids suppresses further endogenous ACTH secretion by the pituitary.

The Epinephrine Test for Pituitary Adrenal Cortical Function

Principle

The anterior pituitary gland is stimulated to secrete ACTH by the administration of epinephrine, and the eosinophil count is followed as a reflection of pituitary adrenal cortical activation. The test affords a means of diagnosing adrenal cortical insufficiency or deficiencies in anterior pituitary ACTH secretion. The epinephrine test may be used as a screening test prior to employing the ACTH test (23).

Procedure

The patient should either be fasting or else the test should be started just prior to a meal. An initial direct count is done (*see* p. 191) and 0.3 mg. of epinephrine is injected subcutaneously or 0.2 mg. given intravenously in 200 cc. of saline over a one-hour period. Four hours after the injection and

before the next meal, another direct eosinophil count is done. The percentage fall during the four-hour test is computed.

Interpretation

A fall of 50 per cent or more in circulating eosinophils excludes the presence of significant primary or secondary adrenal cortical insufficiency.

A negative response at odds with the clinical impression calls for the use of the 4-hour and 48-hour ACTH tests, since it appears that a block in the adrenal cortical activating mechanism may occur both at the hypothalamic and pituitary levels with an essentially normal adrenal cortical reserve present.

Following 25 mg. of ACTH intramuscularly, eosinophils should be depressed 50 per cent of more four hours later in the presence of a normal adrenal cortex (16). In the presence of a marked eosinophilia, the percentile fall in eosinophils may be reduced. In such cases the administration of 10 mg. of ACTH every six hours for forty-eight hours constitutes a useful procedure (48-hour test) (23). It has been found that the 17-ketosteroid excretion during the second day will rise to the upper limit of normal in the presence of adequate adrenal cortical reserve, whereas no increase is found in patients with Addison's disease.

If the susceptibility of the circulating eosinophil is in doubt, one may resort to intravenous administration of 50 cc. of Eschatin in 100 cc. of saline over a 45-minute period. A fall of 40 per cent or more proves the susceptibility of the eosinophils to adrenal cortical hormone and the findings obtained by the epinephrine test are a valid criterion of adrenal cortical function.

Results

The subcutaneous test (Table 9) has been shown to parallel the results obtained by the intravenous test shown in Figure 14. A comparison with the ACTH test reveals good agreement in the group of normal subjects and patients with adrenal insufficiency, whereas there seems to be a somewhat greater impairment in the response to epinephrine in the small group with primary pituitary insufficiency (Fig. 21). Since secondary atrophy of the adrenal cortex follows pituitary insufficiency in man, the value of the ACTH and epinephrine tests as a differential of primary and secondary adrenal insufficiency is limited. In such instances, the measurement of 24-hour urinary 17-ketosteroid excretion before and during the second day of repeated ACTH administration is the test of choice. Using 10 mg. of ACTH intramuscularly every six hours, the level of 17-ketosteroid excretion will reflect the potential adrenal cortical reserve quite closely. Whereas there will be no rise above the control value in the Addisonian, the normal

level will be approached by the patient with hypopituitarism, to an extent dependent on the degree of secondary adrenal cortical involution (24).

Precautions

The use of the epinephrine test is contraindicated in patients with hypertensive and arteriosclerotic heart disease. The dosage of 0.3 mg. should not be exceeded because of the side effects as well as the loss of specificity with large doses.

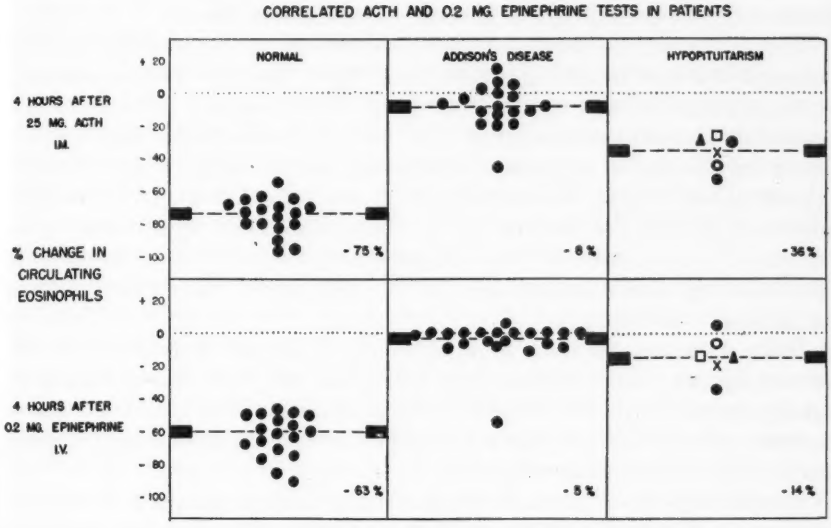

FIGURE 21

(Heavy bars indicate the mean change in the eosinophil level in the group.)

DISCUSSION

In this study, the relationship between the anterior pituitary and the adrenal cortex has been reinvestigated, using the changes in the level of circulating eosinophils as an indicator of adrenal cortical activation.

The over-all functional status of the adrenal cortex is usually reflected by the level of circulating and excreted 11-17 oxysteroids. To date, only this group of steroids, when used in physiologic dosage, has been shown to produce an eosinopenia (10). It is apparent then, that under most circumstances, the change in the circulating eosinophils measures the functional capacity of the adrenal cortex. However, there are known instances of dissociation of cortical function, where an adequate secretion of 11-oxysteroids may be associated with deficiencies in other steroids. In such

rare instances, the eosinophil response will no longer represent the status of the over-all adrenal cortical reserve. Androgenic hyperplasia of the adrenal cortex, seen in the newborn, is an example of such dissociation (25). The eosinophil response to ACTH is adequate, indicating normal 11-17 oxysteroid secretion. There is, however, a marked deficiency in the sodium chloride retaining factors, although the androgenic steroids are produced in excess.

The depression of circulating eosinophils appears to be an unusually sensitive indicator of adrenal cortical activity. In some instances, an eosinopenia may be the only sign of adrenal cortical activation.

The nature of the adrenal cortical control of the level of circulating eosinophils is not readily apparent. It is likely that the adrenal steroids produce a chronic depression of the level of eosinophils, perhaps by increased peripheral destruction of these cells. This depression may be balanced by the rate of eosinophil production and in allergic states is occasionally masked by it. The mechanism by which a constant level of eosinophils is maintained is obscure, but it would seem to be dependent in part on adrenal cortical control. This is suggested by the fact that the eosinophils rise following adrenalectomy and by the constancy of the eosinophil level in Addison's disease.

Daily variations in the eosinophil levels of normal individuals do not exceed 40 per cent variation. The afternoon elevation in eosinophils is analogous to that observed for the lymphocytes by Elmadjian *et al.* These authors related the lymphocyte changes to decreasing adrenal cortical activity as the day progresses (26).

The eosinopenia of stress far surpasses any diurnal variation in the normal state. Although dependent on the integrity of the pituitary-adrenal system, this eosinopenia does not depend on the presence of an intact sympathetic nervous system.

Previous observations on the eosinopenic action of 11-17 oxysteroids (10) have been extended to include commercial aqueous extracts of the adrenal cortex. The relative inactivity of most aqueous extracts as regards the eosinophil depression, and their activation by epinephrine in patients with Addison's disease, probably represents a summation effect of the extract per se and a small ACTH-induced activation of adrenal cortical tissue remnants in response to epinephrine.

The eosinopenic action of ACTH first demonstrated in man (8) has been shown to occur in rat and dog as well. Adrenalectomy abolishes the effect decisively in the dog. In the rat, an eosinopenia will occasionally occur in spite of bilateral adrenalectomy. This is presumed to be because of the presence of accessory adrenal cortical tissue around the ovaries or testes. In the demedullated rat, an eosinopenia regularly occurs with administra-

tion of ACTH. This suggests that the adrenal medulla is not essential for adrenal cortical activation and confirms the study of Simpson *et al.* (27). In addition, it appears that the regenerated adrenal cortices in these animals are capable of producing 11-17 oxysteroids or compounds with related physiologic potentialities.

The eosinopenic action of epinephrine is qualitatively similar to that of ACTH and adrenal cortical hormones. It differs in that its action is dependent upon the presence of adrenal cortex, anterior pituitary and, as shown in the dog, an intact anterior hypothalamus (28). The single observation of Vogt demonstrating an increase in cortical hormone in blood from the adrenal vein of a freshly decapitated dog given epinephrine intravenously suggested a direct action of epinephrine on the adrenal cortex. However, this author subsequently substantiated Long's concept (3) of pituitary mediation of an epinephrine-induced adrenal cortical secretion by observations on rats (5) and more recently on dogs (29). A *pituitary mediated action of epinephrine* has been confirmed by three techniques: the direct measurement of circulating cortical hormone (2), the fall in adrenal ascorbic acid (3) and the production of an eosinopenia.

The degree and timing of the epinephrine-induced and the ACTH-induced eosinopenia are a function of the dosage employed. In man, the administration of more than 0.3 mg. of epinephrine produces a fall in eosinophils of 50 to 70 per cent. This appears to be the maximal response, comparable to that following 10 to 25 mg. of ACTH. The eosinopenia following epinephrine given in 0.2 to 0.3 mg. doses reaches a peak in about four hours. This appears unrelated to the initial eosinophilia, which accompanies a rise in all white cell elements, one half to one hour after the injection (30). Thaddea (31) demonstrated differences in the early leukocyte response following the administration of epinephrine in normal subjects and Addisonians. Since our data evidenced no such differences, it would seem likely that the early changes are not directly referable to adrenal cortical activity, but rather to the hemodynamic status of Thaddea's patients.

The demonstration of a relative refractoriness of the eosinopenic response to epinephrine and to ACTH four to six hours after ACTH or after epinephrine, with a normal response to cortical extract at this time, suggests that a time lag exists between hormonal discharge and resynthesis. It is of interest that following ACTH, ascorbic acid and cholesterol changes (3), as well as radioactive phosphorus turnover rates in the adrenal cortices of animals (32), tend to show maximum changes in four to six hours. The temporal correlation of biochemical changes and eosinopenia is striking.

An eosinopenic response to epinephrine may be obtained in some patients with Addison's disease when doses exceeding 0.5 mg. of epinephrine

are used. Adrenalectomized dogs show no eosinophil fall even with large doses of epinephrine. It is probable that remnants of cortical tissue persist in the Addisonian patient and larger doses of epinephrine may activate these remnants. The ACTH effect may also be prolonged in the absence of the inhibition occasioned by the sharp rise in circulating corticoids seen in normal patients (33). Patients with Addison's disease would thus appear to have adrenal cortices secreting at a maximum rate, but still capable of further stimulation by sufficiently large quantities of endogenous ACTH. This concept lends some support to the "epinephrine treatment" of adrenal insufficiency described by Muirhead in his autobiographic report (34).

In the normal individual epinephrine, used in safe dosage, does not approach the metabolic effectiveness of ACTH. However, the observations of Vogt (2) and of Paschkis *et al.* (35). on the hormone content of adrenal vein blood of dogs, leave no doubt that an increased secretion of adrenal cortical hormone follows epinephrine administration. Unlike Whitelaw (36) we have been unable to demonstrate any marked increase in 17-ketosteroid excretion following the repeated injection of epinephrine over a one to two-day period. However, Hill has found that six hourly injections of 0.5 mg. of epinephrine will lead to a significant rise in 17-ketosteroids after a period of one to three weeks (37). Furthermore, doubling of the 11-oxysteroid excretion in a woman given 0.3 mg. of epinephrine in repeated doses over a 48-hour period is considerably less striking than the five to ten fold increase shown to follow the administration of 40 mg. of ACTH a day (16). Several possible explanations exist for the relative metabolic ineffectiveness of epinephrine in contrast to ACTH. Changes in renal hemodynamics may influence the excretion of electrolytes and uric acid. The marked glycogenolysis following epinephrine administration may alter the pathways of steroid degradation. The dosage may be small in comparison with endogenous sympathetic activity. Finally, the limited action of epinephrine may be associated with the suppression of endogenous ACTH secretion by the rise in the 11-oxysteroid titer. This mechanism has been suggested by Sayers and Sayers (33) for the normal control of ACTH production.

It is possible to produce in dogs anterior hypothalamic lesions which completely prevent any fall in eosinophils following the administration of as much as 1 mg. of epinephrine subcutaneously, though such animals respond normally to ACTH. Eosinopenia follows the injection of an acetone extract of cattle anterior hypothalamus, suggesting the existence of a chemical mediator for the epinephrine-induced pituitary activation (28).

Regulation of pituitary ACTH secretion appears to be of two types: i) the acute type, based on the action of the central nervous system and epinephrine on anterior pituitary ACTH production through a hypothalamic

neurohumoral system, which would represent a phenomenon akin to that described for the secretion of gonadotropins in rabbits (38), and ii) the chronic type of pituitary ACTH regulation, based on the suppressive action of 11-oxysteroids by a mechanism as yet unknown (33).

The use of epinephrine as a test for pituitary-adrenocortical integrity affords a simple, clinical means of demonstrating gross hormonal abnormalities of these glands (12). Laragh and Almy *et al.* (39) using 0.5 mg. of

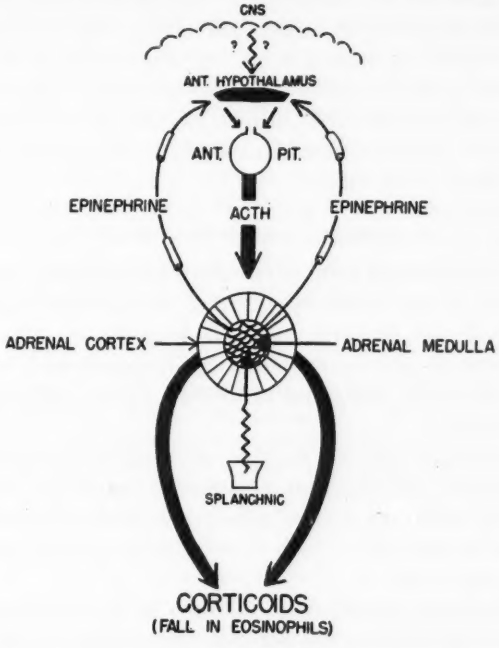

FIGURE 22

epinephrine subcutaneously in adults and Uribe *et al.* (40), using 0.07 mg. of epinephrine per square meter of body surface in children, have developed such tests. Since adrenal insufficiency in man is only relative and since it has been shown that large amounts of epinephrine are capable of stimulating cortical remnants indirectly, it is imperative to keep the dosage at 0.3 mg. subcutaneously or 0.2 mg. intravenously for adults, if adrenal insufficiency is to be diagnosed. Allergic eosinophilia will tend to reduce the eosinopenic response in terms of the percentage fall. Hypopituitarism may, on occasion, be selective as regards the tropic hormones involved. A good eosinopenic epinephrine response does not rule out deficiencies in thyrotropin and gonadotropins.

Both the epinephrine and ACTH (16) tests measure adrenal cortical reserve. In evaluating adrenal function, they appear to be superior to the quantitative determinations of cortical hormone secretion and excretion. The concept of adrenal cortical reserve in various disease states remains to be investigated. The tests are thus necessarily not specific for Addison's disease and hypopituitarism, but afford a measure of prevailing pituitary-adrenal cortical reserve under varying conditions in health and disease.

Epinephrine acts on the eosinophils through the liberation of adrenal cortical steroids (of the 11-17 oxysteroid type). This action is dependent upon an intact chain of stimuli from the anterior hypothalamus to the anterior pituitary and ultimately the adrenal cortex. The diagnostic use of epinephrine in conjunction with cortical extract and ACTH should bring to light numerous breaks in these intimately linked neural, endocrine, and metabolic pathways (Fig. 22).

SUMMARY AND CONCLUSIONS

1. The depression of the level of circulating eosinophils under conditions of stress has been shown to be dependent on an increased pituitary-adrenal cortical activity in rat, dog and man. The magnitude of the eosinophil fall is related directly to the adrenal cortical response and to the potential reservoir of eosinophils, maximal in allergic states and minimal in bone marrow hypoplasia.

2. The eosinopenic response may be obtained in adrenalectomized animals or in patients with Addison's disease with certain aqueous adrenal cortical extracts and with certain adrenal steroids. Compounds E and F will produce an eosinopenia which is not observed with comparable doses of desoxycorticosterone.

3. ACTH produces an eosinophil fall only in the presence of the adrenal cortex and does not require the adrenal medulla for its action on the circulating eosinophil.

4. (a) A single dose of epinephrine leads to an eosinopenic response only in the presence of a normal anterior pituitary ACTH-producing mechanism and an intact adrenal cortex. The anterior pituitary still responds when devoid of its nervous and vascular connections with the hypothalamus. Epinephrine does not appear to be the obligatory activating agent for ACTH production in stress, since significant eosinopenia is still obtained in completely sympathectomized dogs by injection of formaldehyde. (b) Epinephrine has no eosinopenic action per se as shown in adrenalectomized rats and dogs. In patients with Addison's disease, small doses (0.3 mg. or less) of epinephrine given subcutaneously or intravenously, fail to depress eosinophils by more than 30 per cent. However, doses up to 1.5 mg. do lead to depressions approaching the normal fall of 50 per cent. This phe-

nomenon suggests anterior pituitary activation of adrenal cortical remnants. It has been shown that epinephrine may act synergistically with a subliminal quantity of circulating adrenal cortical hormone in the production of eosinopenia. Thus, commercial extracts devoid of an eosinopenic action become activated by the addition of a small, otherwise ineffective dose of epinephrine. (c) The adrenal cortex shows a relative refractory period up to eight hours after a single dose of 25 mg. of ACTH intramuscularly or 0.3 mg. of epinephrine subcutaneously When administered over a period of twenty-four to forty-eight hours, either intravenously or subcutaneously, epinephrine will lead to an eosinopenia but, unlike ACTH, it does not lead to any marked rise in the urinary 17-ketosteroid or 11-oxysteroid excretion. Possible reasons for this self-limited effect are discussed.

5. On the basis of the data obtained, a simple clinical test for the evaluation of pituitary-adrenocortical integrity is presented, based on the four-hour fall in circulating eosinophils following the subcutaneous injection of 0.3 mg. of epinephrine or the intravenous administration of 0.2 mg. in 200 cc. of saline over a one-hour period. A fall exceeding 50 per cent excludes both adrenocortical and pituitary ACTH deficiency.

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REFERENCES

1. Babes, V., and Jonesco, V. M.: Études sur la diminution de la graisse surrénale dans des états pathologiques, *Compt. rend. Soc. de biol.* **65**: 267–269, 1908.
2. Vogt, M.: Observations on some conditions affecting the rate of hormone output of the suprarenal cortex, *J. Physiol.* **103**: 317, 1944.
3. Long, C. N. H., and Fry, E. G.: Effect of epinephrine on adrenal cholesterol and ascorbic acid, *Proc. Soc. Exper. Biol. & Med.* **59**: 67, 1945.
4. Sayers, M. A., and Sayers, G.: Method for the assay of adrenocorticotropic hormone, *Fed. Proc.* **5**: 200, 1946.
5. Vogt, M.: The effect of chronic administration of adrenalin on the suprarenal cortex and the comparison of this effect with that of hexoestrol, *J. Physiol.* **104**: 60, 1945.
6. Selye, H.: The general adaptation syndrome and the diseases of adaptation, *J. Clin. Endocrinol.* **6**: 117, 1946.
7. Dalton, A. J., and Selye, H.: The blood picture during the alarm reaction, *Folia haematol.* **62**: 397, 1939.
8. Thorn, G. W.; Forsham, P. H.; Prunty, F. T. G.; Bergner, R. E., and Hills, A. G.: Clinical studies in Addison's disease, *Ann. New York Acad. Sc.:* **50**: 646, 1949.
9. Hills, A. G.; Forsham, P. H., and Finch, C. A.: Changes in circulating leukocytes

induced by the administration of pituitary adrenocrotocotrophic hormone (ACTH) in man, *Blood* **3**: 755, 1948.

10. Bertelli, H.; Falta, W., and Schweeger, O.: Ueber die Wechselwirkung der Drüsen mit innerer Sekretion ueber Chemotaxis, *Ztschr. f. klin. Med.* **71**: 23, 1910.
11. Godlowski, Z. Z.: Eosinopenia caused by adrenaline and insulin, *Brit. M. J.* **1**: 46, 1948.
12. Recant, L.; Forsham, P. H., and Thorn, G. W.: Observations on the pituitary-adrenal response following epinephrine infusion in man, (abstract) *J. Clin. Endocrinol.* **8**: 589, 1948.
13. Dunger, A.: Eine einfache Methode der Zählung der eosinophilen Leukozyten und der praktische Wert dieser Untersuchung, *München. med. Wchnschr.* **57**: 1910, 1942.
14. Folin, O., and Wu, H.: A simplified and improved method for the determination of sugar, *J. Biol. Chem.* **41**: 367, 1920.:
15. Somogyi, M.: Nitrogenous substances in zinc filtrates of human blood, *J. Biol. Chem.* **87**: 339, 1930.
16. Forsham, P. H.; Thorn, G. W.; Prunty, F. T. G., and Hills, A. G.: Clinical studies with pituitary adrenocorticotropin, *J. Clin. Endocrinol.* **8**: 15, 1948.
17. Kern, A., and Stransky, E.: Beitrag zur kolorimetrischen Bestimmung der Harnsäure, *Biochem, Ztschr.* **290**: 419, 1937.
18. Folin, O., and Wu, G.: A system of blood analysis, *J. Biol. Chem.* **38**: 81, 1919.
19. Callow, H. H.; Callow, R. K., and Emmens, C. W.: Colorimetric determinations of substances containing the grouping -CH²-CO-in urine extracts as an indication of androgen content, *Biochem. J.* **32**: 1312, 1938.
20. Talbot, N. B.; Saltzman, A. H.; Wixom, R. L., and Wolfe, J. K.: The colorimetric assay of urinary corticosteroid-like substances, *J. Biol. Chem.* **160**: 535, 1945.
21. Smith, P. K.: Hypophysectomy and a replacement therapy in the rat, *Am. J. Anat.* **45**: 15, 1930.
22. Thorn, G. W.; Forsham, P. H.: Prunty, F. .T G., and Hills, A. G.: A test for adrenal cortical insufficiency. The response to pituitary adrenocorticotropic hormone, *J.A.M.A.*, **137**: 1005, 1948.
23. Thorn, G. W., with the collaboration of Forsham, P. H., and Emerson, K., Jr.: The Diagnosis and Treatment of Adrenal Insufficiency. *in* American Lecture Series in Endocrinology edited by Willard O. Thompson, Springfield, Illinois, Charles C Thomas, Publisher, 1949.
24. Thorn, G. W., and Forsham, P. H.: Metabolic changes in man following adrenal and pituitary hormone administration, *in* Recent Progress in Hormone Research, Vol. 4, New York, Academic Press, Inc., 1949, p. 224.
25. Wilkins, L.; Fleischmann, W., and Howard, J. E.: Macrogenitosomia precox associated with hyperplasia of the androgenic tissue of the adrenal and death from corticoadrenal insufficiency: a case report, *Endocrinology* **26**: 385, 1940.
26. Elmadjian, F., and Pincus, G.: A study of the diurnal variations in circulating lymphocytes in normal and psychotic subjects, *J. Clin. Endocrinol.* **6**: 287, 1946.
27. Simpson, M. E.; Li, C. H., and Evans, H. M.: Effect of adrenocorticotropic hormone (ACTH) on hypophysectomized adrenal-demedullated rats, *Proc. Soc. Exper. Biol. & Med.* **60**: 329, 1945.
28. Hume, D. M.: The role of the hypothalamus in the pituitary-adrenal cortical response to stress, (abstract) *J. Clin. Investigation* **28**: 790, 1949.
29. Vogt, M.: Personal communication.
30. Lucia, S. P.; Leonard, M. E., and Falconer, E. H.: The effect of the subcutaneous

injection of adrenalin on the leukocyte count of splenectomized patients and of patients with certain diseases of the hematopoietic and lymphatic systems, *Am. J. M. Sc.* **194:** 35, 1937.

31. THADDEA, S.: Nebennierenrinde und Leukocyten regulation, *Med. Welt* **12:** 123, 1938.
32. GEMZELL, C. A.: The effect of corticotrophic hormone and oestrogen on liver glycogen content and phosphate metabolism in the adrenal cortex, *Acta Endocrinologica* Suppl. 1 to Volume 1, 1948.
33. SAYERS, G., and SAYERS, M. A.: Regulation of pituitary adrenocroticotrophic activity during the response of the rat to acute stress, *Endocrinology* **40:** 265, 1947.
34. MUIRHEAD, A. L.: An autograph history of a case of Addison's disease, *J.A.M.A.* **76:** 652, 1921.
35. PASCHKIS, K. E..; CANTAROW, Z.; WALKING. A. A.; PEARLMAN, W. H.; RAKOFF, A. E., and BOYLE, D.: Secretion and excretion of CHO-active adrenal compounds (oxysteroids), *Fed. Proc.* (Amer. Physiol. Soc.) **7:** (Part I): 90, March 1948.
36. WHITELAW, M. J.: Pseudohermaphrodism: early and late recognition, (abstract) *J. Clin. Endocrinol.* **8:** 583, 1948.
37. HILL, S. R., *et al.:* Personal communication.
38. HARRIS, G. W.: The induction of ovulation in the rabbit, by electrical stimulation of the hypothalamohypophysial mechanism, *Proc. Roy. Soc., London,* A. B. **122:** 384, 1937.
39. LARAGH, J. H., and ALMY, T. P.: Changes in circulating eosinophilis in man following epinephrine, insulin, and surgical operations, *Proc. Soc. Exper. Biol. & Med.* **69:** 499, 1948.
40. URIBE, R. C.; ZYGMUNTOWICZ, A. S.; WOOD, M.; CHRISTO, E., and TALBOT, N. B.: Observations on adrenal cortical "sugar-fat-nitrogen" hormone production as measured by urinary 11-17-oxysteroid-like substances and by counts of circulating eosinophils, *Tr. Am. Ped. Soc.* 1949 (in press).

CLINICAL EXPERIENCE WITH A BIOASSAY METHOD FOR THE DETERMINATION OF URINARY CORTICOSTEROIDS*

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NORMAL human urine contains measurable amounts of the adrenal cortical hormone which promotes gluconeogenesis and which, by promoting the conversion of body protein to carbohydrate, maintains the blood glucose and liver glycogen levels in the fasting state. In 1942 Reinecke and Kendall (1) published a method for the bioassay of this adrenal cortical hormone which made use of the rapid loss of liver glycogen in the fasted adrenalectomized rat as compared with the adrenalectomized animal treated with adrenal cortical extract. This method was modified and adapted to the mouse and used for testing urinary extracts in clinical investigation by Eggleston, Johnston and Dobriner (2), Venning, Kazmin and Bell (3) and Dorfman, Ross and Shipley (4).

The method used in this laboratory is that of Eggleston, Johnston and Dobriner with a few minor changes. A comparison of the techniques used and the results obtained with these methods is given in detail in the Transactions of the Tenth Conference on Metabolic Aspects of Convalescence Including Bone and Wound Healing (5).

METHOD

Preparation of urinary extracts

For assay at the usual two levels (*see* subsequent sections) a 48-hour urine collection is obtained. The urine is acidified to pH 1 with concentrated sulfuric acid, allowed to stand for twenty-four hours in the icebox, and extracted continuously for forty-eight hours at room temperature with 400 cc. of peroxide-free ether (Mallinckrodt U.S.P. XII) to each 1500 cc. of urine. The continuous extraction apparatus was made by Scientific Glassware Apparatus Company, Bloomfield, New Jersey, according to specifications furnished us by Dr. Dobriner. The ether extract is transferred to a one-liter separatory funnel and washed twice with 30 to 50 cc. of 2N NaOH, once with 30 to 50 cc. of 1N

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HCl and twice with 20 cc. of distilled water. The alkali, acid and water washings are back extracted twice with 150 cc. to 200 cc. portions of fresh ether. The ether extracts are pooled and allowed to stand for at least thirty minutes. Any water which separates is drawn off. The extract is then filtered through heavy filter paper (Schleicher and Schuell #598) into a one-liter Erlenmeyer flask and evaporated to about 50 cc. on a steam bath. It is next transferred to a 50 cc., long-necked, flat-bottomed boiling flask, returned to the steam bath and evaporated to a volume of 5 or 6 cc. This last amount is taken to dryness under reduced pressure in a water bath at a temperature of 45° to 50° C; 3 cc. of benzene is added and the extract again taken to dryness under reduced pressure and further dried overnight in a vacuum dessicator. The dry extract is stored at room temperature until used for assay.

Preparation of extract for injection

Twenty-four hours before assay, 0.2 cc. of absolute alcohol is added to the dry extract; the flask is stoppered and allowed to stand for two to three hours; at the end of that time 1.05 cc. of purified sesame oil is added. The oily extract is transferred to a 15 cc. conical tipped centrifuge tube by inverting the flask over the tube and allowing to drain for an hour. Two tenths of a cc. of absolute alcohol and 1.05 cc. of sesame oil are added to the flask as before; this second transferral is allowed to drain overnight in the ice box. The alcohol rises to the surface of the oil and evaporates.

Assay procedure

Male mice of a uniform strain, and weighing from 20 to 25 grams are used. Before adrenalectomy they are maintained on a regular diet of "Purina Lab Chow."[1] The adrenals are removed under nembutal (1 mg./10 Gm. body weight) and ether when necessary. On awakening, each mouse is given 0.25 cc. of Adrenal Cortex Extract (Upjohn) subcutaneously and placed in an individual cage containing a generous amount of cotton. The cages are kept in a constant temperature room maintained at 80° ±3° F. The mice are given 0.9 per cent saline as drinking water and an excess of "Diet A" is provided. This diet is the McCollum Lactation Diet (6) and contains:

1215	Gm. whole wheat flour
270	Gm. casein
180	Gm. Klim (powdered whole milk)
27	Gm. calcium carbonate
18	Gm. sodium chloride
90	Gm. butter substitute
1	per cent cod liver oil

At 5 P.M. on the fourth postoperative day "Diet B" is substituted for "Diet A." "Diet B" is identical to "Diet A," except that 1215 Gm. of "Re-Knu Health Bran" is substituted for the wheat flour. The reason for this change will be discussed later. At 8 A.M. on the fifth postoperative day the mice are transferred to clean cages containing cotton but no food or water. The mice are injected at 8:30 A.M., 10:30 A.M. and 12:30 P.M. with 0.2 cc.; and at 2:30 P.M. with 0.1 cc. of the extract being tested. They are weighed after the last injection. At 3:00 P.M., the animals are killed by breaking their necks; the livers are quickly removed (within fifty seconds) and dropped into 50 cc

[1] Purina Mills, St. Louis, Missouri.

centrifuge tubes containing 5 cc. of 30 per cent KOH, which have been heating in a boiling water bath for thirty minutes or more. The tubes are agitated every five minutes for twenty-five minutes, removed from the bath, and 5.7 cc. of 95 per cent alcohol (made up from absolute alcohol at least once a week) is added to each tube. The mixture is shaken, returned to the water bath and brought to a boil. The tubes are then removed and allowed to cool. The amount of flocculent precipitate (glycogen) which appears at this time permits estimation of the size of the aliquot needed for the sugar test. In fact in every instance in which only a scant precipitate appeared, the corticosteriod assay gave negative results. The tubes are centrifuged at high speed for fifteen to twenty minutes: the supernatant liquid is decanted and the tubes are inverted on paper-toweling to drain. They may stand this way overnight. On the following morning 2.5 or 4.0 cc. of N H_2SO_4, depending on the amount of precipitate which was observed, is added to each tube; the tubes are stoppered with tear bulbs and the glycogen digested in a boiling water bath for two to two and a half hours. After cooling, 0.5 cc. of 5N NaOH is added to those which received 2.5 cc. of H_2SO_4, and 1.0 cc. of 5N NaOH is added to those which received 4.0 cc. Those not expected to contain much sugar are restored to 2.5 cc. volume with 1.8 N Na_2SO_4, whereas those which showed a very heavy precipitate of glycogen are diluted to a volume of 10, 20 or even 40 cc. with the same solution. Analyses for sugar are made by Nelson's modification (7) of the Somogyi-Shaffer-Hartman method.[2]

Calculations

Five mice are used at each dose level, each mouse receiving a total dose of 0.7 cc. of oily extract. The test result is considered positive if 3 or more of the 5 mice show more than 0.9 mg. of liver glycogen per 10 Gm. of body weight. A mouse unit is defined as the least amount of hormone which, when injected into each of 5 mice will give a positive response. Therefore, to test for 24 mouse units/24 hrs. it is necessary to take from the oily extract the equivalent of five hours' worth of urine or, in a 48-hour collection, 5/48 of 2.1 cc., *i.e.*, about 0.2 cc. (*see* preparation of urinary extract) and make it up to a volume of 3.5 cc. (0.7 cc. for each of 5 mice) with sesame oil. If a positive result is obtained, the urine can be said to contain at least 24 mouse units/24 hrs. The remaining forty-three hours' worth of urine divided among 5 mice is just sufficient to test for 2.8, or, in round numbers, 3.0 m.u./24 hrs.

DISCUSSION OF THE METHOD

Extraction

There is reason to think that the method of extraction is efficient and reliable. Lieberman (8) states that synthetic steroid sulfates can be almost quantitatively extracted by this method. Engel (9) found more reducing steroids in an extract prepared by us than in an extract of the same urine sample made by the method of Heard, Sobel, and Venning (10). Talbot (11) agrees that the extraction by this method is more complete than by that of Talbot, Saltzman, Wixom and Wolf (12) but feels that it also yields nonspecific substances which interfere with the chemical assay.

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[2] The authors now believe that a much less exact determination of the sugar would suffice, since the mice do not vary greatly in weight and it is necessary to know only whether there is more or less than 0.9 mg. glycogen/10 Gm. body weight.

Storage

The experience of other laboratories has suggested that, although extracts dissolved in oil might deteriorate, ones stored in the dry state might be kept for long periods. Some chance observations suggest that this may not be true. Thus, an extract from a normal individual gave negative results for 2.8 m.u./24 hrs. after two months' storage. A nine-month-old extract from a patient with Cushing's syndrome, previously found to excrete more than 48 m.u./24 hrs., gave negative results for 12 m.u./24 hrs. An extract of pregnancy urine was found to contain more than 48 and less than 96 m.u./24 hrs. when stored for ten days in the dry state, but less than 48 m.u. when stored for 120 days. Another extract of the same pool, stored for ten days in oily solution, was also negative for 48 m.u./24 hrs. A sample collected one week later from the same patient gave negative results for 12 m.u./24 hrs. after forty days' storage in the dry state. These findings cast doubt on any negative response obtained with an extract which has been stored for a month or more. It is possible that storage at low temperatures might prevent some of this loss.

Preparation of the oily extract

The method of dissolving the urine extract in a very small volume of sesame oil and removing the same quantitatively from the flask is admittedly clumsy and time-consuming. Larger quantities however offer difficulties in injection and may leak out from the point of puncture or, if retained, fail to be absorbed.

Preparation of the mice

By far the most troublesome part of the entire procedure, and that which may most easily lead to error, is the postoperative care and feeding of the mice. In all our earlier tests, control mice, injected with sesame oil alone, were used each time. "Positive controls," that is fasted-adrenalectomized-sesame oil-injected animals showing more than 0.9 mg. of liver glycogen per 10 Gm. of mouse, occurred not infrequently at the start of the work when "Diet A" was fed throughout. This diet is higher in carbohydrate and lower in roughage than "Diet B." At autopsy it was found that the intestinal tract still contained considerable food. If the fast was made much longer, however, some of the animals would perish and some would be too nearly moribund to react. By substituting a diet containing more bran for the last fifteen hours before the fast, more rapid emptying of the intestinal tract occurred and "positive" controls were rare. Figure 1 shows the effect of variations in the diet on this phenomenon. Thus, there is a narrow margin between a fast sufficiently rigorous to lower liver glycogen and one

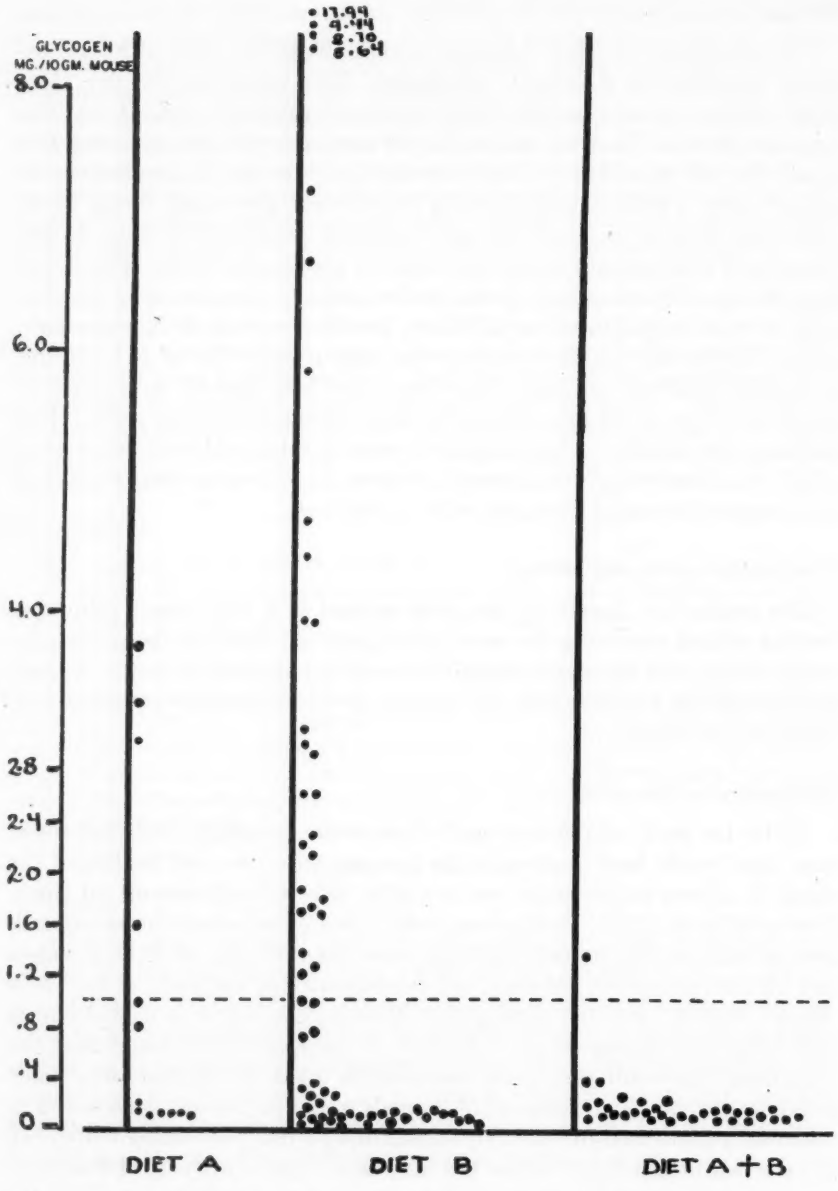

FIG. 1. Effect of diet on liver glycogen of "control" mice.

so rigorous as to debilitate the animal. The mice were found to be also very sensitive to temperature changes and to toxic substances in certain of the urinary extracts. A variation in room temperature of more than 3 degrees from 80 degrees often resulted in dead or unreactive animals. Less than 5 mice per dose level was found unsatisfactory, owing to individual variations in responsiveness. The range of values in control adrenalectomized animals from which the criteria for a positive response were derived, is shown in Figure 1. Controls were not used routinely after this range had been established.

Interpretation

No dose response curve made with pure steroids was obtained. Although a standard adrenal cortical extract (Upjohn) was titrated, results in patients were not expressed in these terms. The results were expressed in mouse units per twenty-four hours and evaluated by comparison with the range found in normal individuals.

Comparison with chemical methods

In spite of the difference in the nature of the tests and the units employed a considerable degree of parallelism exists between the chemical test of Talbot, Saltzman, Wixom and Wolf (12) and the biologic test here reported.

RESULTS

Normal individuals

Eighteen assays were made on 15 normal males and 14 assays on 10 normal females. These are shown in Table 1.

In all except 1 normal individual the results were positive for 2.8 m.u./24 hrs. In all except 2 individua's they were negative for 24 units; both of these subjects showed negative results when tested a second time. Out of 9 individuals tested for 12 units, only 1 gave a positive response. From these data it is concluded that values consistently above 24 or below 2.8 m.u./24 hrs. are abnormal; indeed, a single value outside this range is very likely to be abnormal.

Addison's disease

Table 2 summarizes the results in Addison's diasese. If the test, as we believe, is specific for adrenal cortical hormone, it should be at low levels in these patients. In several cases, therefore, assays were carried out at levels of less than 2.8 m.u./24 hrs. At no level was a positive response obtained in an untreated patient. One patient, who when not receiving treatment showed a negative response for 1.4 m.u./24 hrs., showed a positive

TABLE 1. RESULTS IN NORMAL SUBJECTS

Males					Females				
Subject	Mouse units per 24 hours				Subject	Mouse units per 24 hours			
	2.8	6	12	24		2.8	6	12	24
R.Be			0		C.A.	+		0	
R.Br	+			0	D.B.	+		0	
J.C.	+			0	D.B.	+			0
P.C.	+			0	E.C.	+			
F.D.	+			0	E.C.	+		0	
W.J.	+			0	E.C.	+			0
B.L.	+	+			J.G.	+		0	
A.P.	+			0	G.M.	+			0
M.P.			0		E.O.	+			0
E.R.	0				M.P.	+			0
E.S.	+		0		M.T.	+			+
E.S.	+			0	M.T.	+			0
J.S.	+		+		P.W.	+			
B.T.	+			+	M.W.	+			0
B.T.	+			0					
F.W.			0						
F.W.			0						
D.W.	+			0					

response for 3 m.u./24 hrs. in the first twelve hours after receiving 100 cc. of adrenal cortical extract.

Cushing's syndrome

It is our belief that the manifestations of this syndrome are chiefly due to an overproduction by the adrenal cortex of the gluconeogenic hormone, which this test is designed to measure. Eight classic cases of Cushing's syndrome were tested (Table 3). Three of these patients had a benign tumor of one adrenal, 3 did not have a tumor, and in 2 the adrenals were not surgically explored. All 8 excreted at least 24 m.u./24 hrs. One patient with tumor excreted at least 60, and 2 of the non-tumor patients, at least 96 m.u./24 hrs. In 2 more cases (E.M. and M.H.), thought to be in remission, the test in the first gave positive results for 12, and negative for 24 m.u./24 hrs.; and in the second, positive results for 24, and negative for 48 m.u./24 hrs. Patient R.D. entered the hospital with unmistakable Cushing's syndrome but shortly afterward developed signs and symptoms of adrenal insufficiency and died. The low assay (negative for 3.25 m.u./24 hr.) agreed with the clinical picture and with the low 11-oxysteroids.

In summary, the urinary corticosteroids were well above normal in all the active cases of Cushing's syndrome.

TABLE 2. RESULTS IN ADDISON'S DISEASE

Patient, MGH unit #	Sex, Age (yrs.)	Important features	Corticosteroid assay m.u./24 hrs.	
			Positive for	Negative for
1. A.A. 487800	M 45	History of tuberculous epididymitis and nephrectomy for renal tuberculosis; admitted for nephrolithiasis and pyelonephritis; found to have weakness, weight loss, pigmentation and hypertension; 17-KS, 3.2 mg./24 hrs.	—	1
2. R.D. 485460	F 60	Admitted in 1945 with classic Addison's disease; maintained in fair condition with DOCA pellets but had frequent attacks of hypoglycemia. Died suddenly at home in 1947.	—	1.5
3. S.G. 385496	F 49	Classic Addison's disease; rheumatoid arthritis; maintained on DOCA and testosterone pellets. Received 100 cc. adrenal cortical extract intravenously on day of first assay.	3 — —	— 6 6 1.4
4. H.H. 474136	F 26	Weakness, hypotension; pigmentation; absent axillary hair; improvement on DOCA; 17-KS, 0.9 mg./24 hrs. Died suddenly at home.	—	0.75
5. A.J. 366564	M 38	Classic Addison's disease, with history of tuberculosis; maintained on DOCA; insulin tolerance test showed hypoglycemia unresponsiveness.	—	1.7
6. E.H. 258599	M 41	Admitted eight yrs. before with weakness, weight loss and pigmentation; maintained in excellent condition with small amounts of DOCA; insulin tolerance test showed hypoglycemia unresponsiveness.	—	3
7. M.K. 209947	F 51	Classic Addison's disease for six yrs.; maintained on DOCA; insulin tolerance test showed hypoglycemia unresponsiveness.	—	1.5
8. M.M. 593088	F 34	Early Addison's disease confirmed by lack of response to adrenocorticotropic hormone; 17-KS, 0.5 mg./24 hrs.	—	1.3
9. E.M.	F 49	History of renal tuberculosis; classic Addison's disease; improvement on DOCA.	—	1.0

Acromegaly

Insulin resistance is a constant finding in acromegaly. Indeed, it is more marked in this condition than in Cushing's syndrome. Of 8 patients with acromegaly (Table 4), all of whom were resistant to insulin, only 1 excreted

TABLE 3. RESULTS IN CUSHING'S SYNDROME

Patient MGH unit #	Sex, Age (yrs.)	Important features	Corticosteroid assay m.u./24 hours	
			Positive for	Negative for
1. B.A. 567276	F 33	Weakness and back pain for five yrs.; amenorrhea for thirteen mos.; facies of Cushing's syndrome, obesity, plethora, bruises, hypertension, osteoporosis of the spine; 17-KS, 5 to 10 mg./24 hrs.; diabetic type of glucose tolerance curve; insulin resistant. Adrenal tumor removed; died 16th postoperative day.	60 48 48	120 96 96
2. L.C. 592211	M 63	Diabetes mellitus for twenty-five yrs.; osteoporosis for two yrs.; facies of Cushing's syndrome; obesity, plethora, bruises, hypertension; required large amounts of insulin; receiving methyltestosterone.	24 24 24	 48 48
3. C.D. 562033	M 31	Weakness, obesity, hypertension; facies of Cushing's syndrome; 17-KS, 23 to 44 mg./24 hrs.; insulin resistant. Operation disclosed bilateral adrenal hyperplasia. The first two assays were done during a period of no treatment; the second two, during testosterone therapy; and the fifth, when testosterone was omitted and ten days after resection of $\frac{1}{3}$ of left adrenal.	24 48 48 48 96	— — 96 96 144
4. A.D. 499922	F 20	Classic Cushing's syndrome of two years' duration; 17-KS, 25 mg./24 hrs.; insulin resistant. Operation disclosed bilateral adrenal hyperplasia.	24 24	48 48
5. I.G. 350260	F 13	Failure to grow, obesity and back pain since 11 yrs. of age; Appearance of Cushing's syndrome, hypertension, osteoporosis, 17-KS, 13 mg./24 hrs.; diabetic type of glucose tolerance curve; insulin resistant. Remission following x-ray treatment to pituitary; recurrence two years later; operation disclosed bilateral adrenal hyperplasia.	48 96	96 —
6. J.D. 610	F 37	Rapidly progressive, severe osteoporosis; appearance of Cushing's syndrome; hypertension; 17-KS, 1.7 mg./24 hrs.; diabetic type of glucose tolerance curve. Recovery following removal of an adrenal tumor.	24	48
7. S.W. 619603	F 33	Marked arteriosclerosis with symptoms of intermittent claudication for thirteen years; irregular periods and obesity for three years; round face, easy bruisability, striae, hypertension. Adrenal tumor found at operation.	24 24	48

TABLE 3—(*continued*)

Patient, MGH unit #	Sex, Age (yrs.)	Important features	Corticosteroid assay m.u./24 hrs.	
			Positive for	Negative for
8. R.S. Dr. H.S. Guterman's pt.	F 33	Obesity; amenorrhea; hirsutism; easy bruisability; osteoporosis; insulin resistance.	24	48
9. E.M. 397957	F 51	Had had Cushing's syndrome for two years, with osteoporosis, obesity, hypertension, diabetes, slight insulin resistance. In remission since x-ray treatment pituitary and removal of one hyperplastic adrenal at that time.	12	24
10. M.H. 482838	F 33	Admitted for sympathectomy for severe hypertension; history of obesity, red face, easy bruisability. Four years previously relieved by x-ray treatment to pituitary. Operation disclosed an adrenal tumor which had degenerated and became hyalinized.	6, 24	48
11. R.D. 546578	M 38	Osteoporosis, hypertension, weakness, and appearance of Cushing's syndrome; roentgenogram suggestive of tumor over right kidney. 17-KS, 3 mg./24 hrs.; insulin tolerance test normal. Ten days after corticosteroid assay, went into collapse resembling Addisonian crisis in which he died. 11-OCS, which had been .91 mg./24 hrs. fell to 0.7 mg./24 hrs. at this time.	—	3.25

as much as 24 m.u./24 hrs. It would appear, therefore, that the insulin resistance of acromegaly is due to the direct action of a pituitary hormone and not, as in Cushing's syndrome to an increased excretion of adrenal cortical hormone (13). It may be explained by the finding of Fishman, Wilhelmi and Russell (14) that crystalline growth hormone is powerfully glycostatic.

If this test is an index of the production of ACTH in acromegaly, there is no correlation between the production of ACTH and that of FSH. Thus of 3 patients who excreted less than 3 m.u./24 hrs. of corticosteroid, the FSH was normal in 2; and of the 4 patients in whom FSH excretion was low, the corticosteroids were normal in 3. It follows that if the low level of corticosteroid excretion means encroachment on the cells producing ACTH, then this hormone is probably not elaborated by the same cells (basophil cells) as FSH.

TABLE 4. RESULTS IN ACROMEGALY

Patient, MGH unit #	Sex, Age (yrs.)	Important features	Corticosteroid assay m.u./24 hrs.	
			Positive for	Negative for
1. M.C. 321288	F 40	Entered in 1941 for breast abscess related to galactorrhea; acromegalic facies noted; sella turcica enlarged by x-ray examination; normal pregnancy in 1945; FSH normal; 17-KS, 6.3–7.2 mg./24 hrs.; glucose tolerance test normal; insulin tolerance test showed insulin resistance.	3	24
2. R.R. 575709	F 47	Acromegaly for ten years; recent hypertension; receiving 10 mg. of stilbestrol daily at time of essay; FSH pos. for 96 m.u./24 hrs., prior to stilbestrol treatment; 17-KS, 6.4 mg./24 hrs.	—	3, 24
3. R.V. 48511	F 29	Entered for amenorrhea and persistent lactation; features acromegalic. FSH pos. for 3 m.u./24 hrs., neg. for 6 m.u./24 hrs.; 17-KS, 16 mg./24 hrs.; insulin tolerance test showed insulin resistance.	—	3, 24
4. C.N. 11134	M 30	Mild acromegaly for five years; FSH pos. for 96 m.u./24 hrs.; 17-KS, 12.2 mg./24 hrs.	3	24
5. J.M.	M 29	Acromegalic features for eight years; FSH pos. for 6.5 m.u./24 hrs., neg. for 52 m.u./24 hrs.; 17-KS, 7.7 mg./24 hrs.; slight insulin resistance.	24	—
6. D.S.	F 29	Amenorrhea; sella turcica enlarged by x-ray examination; FSH neg. for 6.5 m.u./24 hrs.; 17-KS, 11.8 mg./24 hrs.; first assay, control; second assay, during therapy with 30 mg. of stilbestrol daily.	3 3	24 24
7. C.S. 347319	F 21	Unmistakable acromegaly; amenorrhea; acne; slight hirsutism; FSH pos. for 7 m.u./24 hrs., neg. for 10 m.u./24 hrs.; insulin tolerance test showed insulin resistance. Last assay, during stilbestrol therapy.	3 3, 6 6, 12 ?6	— — — —
8. N.M.	F 59	Unmistakable acromegaly for ten years; FSH pos. m.u./24 hrs., neg. 26 m.u./24 hrs.; 17-KS, 9.9 mg./24 hrs.; insulin tolerance test showed insulin resistance.	?3 3	24 24

Hypopituitarism

All 13 patients in Table 5 had some form of hypopituitarism. The first 8 had classic panhypopituitarism, *i.e.*, deficiency of all the hormones of

the anterior lobe. In Cases 9 and 10 the patients had all the findings of panhypopituitarism except that the FSH tests were not low; both had x-ray evidence of pituitary adenoma. In Cases 11 and 12 the findings were those of panhypopituitarism except that the subjects did not exhibit hypogly-

TABLE 5. RESULTS IN HYPOPITUITARISM

Patient, MGH Unit #	Diagnosis	Sex, Age (yrs.)	Important features	Corticosteroid assay m.u./24 hrs.	
				Positive for	Negative for
1. F.H. 68854	Pituitary tumor; panhypopituitarism	M 59	Known to have a pituitary tumor for fifteen years; weakness and impotence recently; FSH positive for 3 m.u./24 hrs., neg. for 6.5 m.u./24 hrs.; 17-KS, 0.6 to 1.5 mg./24 hrs.; insulin tolerance test showed hypoglycemia unresponsiveness.	—	3, 24
2. M.H. 568954	Panhypopituitarism	F 56	Appearance and laboratory findings of myxedema; also low serum Na; high serum K; FSH weakly pos. for 6.5 m.u./24 hrs.; 17-KS, 1.6 mg./24 hrs.; insulin tolerance test showed hypoglycemia unresponsiveness.	— — —	2.8 2.8 3.25
3. H.J. 550872	Chromophobe adenoma; panhypopituitarism	M 44	X-ray evidence of pituitary tumor; FSH neg. for 6.5 m.u./24 hrs.; 17-KS, 1.7 mg./24 hrs.; insulin tolerance showed hypoglycemia unresponsiveness.	— — —	1.5 1.5 3
4. R.J. 310781	Pituitary dwarfism	M 22	Ht. 4'5"; never developed sexually; FSH neg. for 3 m.u./24 hrs.; 17-KS, 1.3 mg./24 hrs.; has episodes suggestive of hypoglycemia.	— —	1.7 2.4
5. H.S. 512466	Panhypopituitarism	F 43	Weight loss, weakness, amenorrhea for twenty-one years; appearance of myxedema; BMR −36 per cent; FSH. pos. for 6.4 m.u./24 hrs., neg. for 13 m.u./24 hrs.; 17-KS, 0.7 mg./14 hrs.	—	1.5
6. M.W. 160451	Panhypopituitarism; Sheehan's syndrome	F 39	Classic panhypopituitarism since difficult delivery eighteen years before; FSH pos. for 3 m.u./24 hrs.; 17-KS, 1.0 mg./24 hrs.; BMR's −23 and −38 per cent; insulin tolerance test showed hypoglycemia unresponsiveness.	— —	3 3
7. M.W. 614303	Panhypopituitarism; Sheehan's syndrome	F 32	Weakness, lassitude, and amenorrhea since difficult delivery four years before; FSH neg. for 3 mg./24 hrs.; 17-KS, 1.6 mg./24 hrs.; BMR −38 per cent; insulin tolerance test showed hypoglycemia unresponsiveness.	—	3
8. M.T. 488329	Panhypopituitarism	F 61	Admitted for myxedema; BMR −45, cholesterol 625 mg. per cent; FSH pos. for 6.5 m.u./24 hrs.; Went into collapse with an attack of pyelonephritis; good response to adrenal cortical extract; thyroid and testosterone. On thyroid at time of assay.	—	1.5
9. J.A. 257643	Pituitary tumor, ? type	M 33	Pallor and sluggishness; sella turcica enlarged; hypoglycemic coma followed tooth extractions; FSH neg. for 6.5 m.u./24 hrs.; 17-KS 2.1, 3.6 mg./24 hrs.; BMR −50; insulin tolerance test showed hypoglycemia unsponsiveness.	3	24
10. M.B. 288692	Pituitary tumor, ? type	M 68	Weakness 20 yrs.; eunuchoid appearance; sella turcica enlarged; BMR −23; 17-KS 21 mg./24 hrs.; strong evidence for pituitary function was FSH. pos. for 26 m.u./24 hrs.; neg. for 52 m.u./24 hrs.; insulin tolerance test showed hypoglycemia unresponsiveness.	—	3, 24
11. N.B.	? panhypopituitarism; ? suprasellar syndrome	M 46	Short stature, absent sexual development, obesity, headache; FSH neg. for 6.5 m.u./24 hrs.; 17-KS 0.5 mg./24 hrs.; P.B.I. 2.7 per cent; Kepler water test positive; insulin tolerance test shows hypoglycemia unresponsiveness.	—	1.5
12. L.D.	? panhypopituitarism; ? suprasellar syndrome	M 35	Weakness, drowsiness, loss of libido and episodes suggestive of hypoglycemia; FSH neg. for 3 m.u./24 hrs.; 17-KS 2.0, 4.6 mg./24 hrs.; testicular biopsy showed hypo-spermatogenesis and absent Leydig cells; insulin tolerance test normal.	—	1.0
13. S.C.	Eunuchoidism "with negative FSH."	M 18	Infantile genitalia, no beard, high voice, absent axillary hair, absent abdominal hair; delayed bone age; immature testis on biopsy; FSH neg. for 6.5 mg./24 hr.; 17-KS 3.1 mg./24 hrs.	—	1.5

cemia unresponsiveness when given an insulin tolerance test. In Case 13 the patient had "eunuchoidism-with-negative-FSH," presumably with deficiency of pituitary gonadotropins without deficiency of other anterior lobe hormones.

Of the 13 patients, all except Case 9 excreted less than 3 m.u./24 hrs. Of 6 tested at lower levels (1.0 to 1.7 m.u./24 hrs.), all gave negative results. In Case 8 the patient was thought to have primary myxedema, until an acute infection precipitated an Addisonian crisis, which responded to adrenal cortical extract. This patient is to be compared with the patient in Case 13, Table 7, who was thought to have panhypopituitarism until laboratory tests showed an FSH excretion greater than 96 m.u./24 hrs. and a corticosteroid assay of more than 3 m.u./24 hrs. A diagnosis of primary hypothyroidism was made and confirmed by the response to thyroid therapy.

Adrenogenital syndrome

Four females with virilism due to adrenal hyperplasia were studied (Table 6). Three excreted less than 3 m.u./24 hrs.; the fourth was not tested for less than 24 m.u./24 hrs. but the response was negative at that level.

The 17-ketosteroid excretion, as expected, was markedly elevated in all 4 patients. This finding is consistent with the clinical evidence of increased androgen production and increased protein anabolism. Steroids causing gluconeogenesis were not expected to be present in excess. Since females with the adrenogenital syndrome do not show any signs of adrenal insufficiency, and since the "11-oxycorticosteroid" excretion of the patients in Table 6 and of others reported by Talbot, Albright, Saltzman, Zygmuntowicz and Wixom (15) was not low as measured by their chemical test, it came as a surprise to find the corticosteroids lower than normal by the biologic assay. Moreover, patient C. O. (Case 1, Table 6) continued to have low corticosteroid assays while receiving ACTH injections (Table 8). The possibility was considered that the urinary extracts from these patients might contain some androgen and that this might inhibit gluconeogenesis in the mouse. To test this hypothesis, an assay of a urinary extract known to be positive for 3 m.u./24 hrs. was repeated with the addition of 1 mg. testosterone propionate to the solution injected in each animal. The result was unchanged. The low corticosteroid excretion by biologic assay in adrenal virilism remains unexplained.

Miscellaneous conditions

In Table 7, it may be seen that Patients 1, 2 and 3 had severe idiopathic osteoporosis. In contradistinction to the findings in Cushing's syndrome,

TABLE 6. RESULTS IN ADRENOGENITAL SYNDROME

Patient, MGH unit	Sex, Age (yrs.)	Important features	Corticosteroid assay m.u./24 hrs.	
			Positive for	Negative for
1. C.O. 169536	F 12	Classic adrenogenital syndrome, apparent since age of 5 yrs.; precocious skeletal growth and maturation; genital malformation as described by Young; 17-KS, 52 to 75 mg./24 hrs.; 11-OCS, 0.24 to 0.29 mg./24 hrs.	— — —	3 3 3
2. E.F.	F 30	Classic adrenogenital syndrome similar to preceding case; 17-KS, 30 to 80 mg./24 hrs.; 11-OCS, 2.4 mg./24 hrs.	—	3
3. M.W.	F	One of two sisters with marked virilism since early childhood 17-KS, 30 to 100 mg./24 hrs.; 11-OCS, 2.4 mg./24 hrs.	—	24
4. T.M. 586528	F 8	Weight and bone age that of 12-year-old girl; enlarged clitoris; genitalia not otherwise abnormal; pubic hair; acne; 17-KS, 9.0 mg./24 hrs.; 11-OCS, 0.13 and 0.12 mg./24 hrs. Operation disclosed hyperplastic adrenals.		3

in which osteoporosis also occurs, the assays were not elevated. Patients 4 and 5 with senile and/or postmenopausal osteoporosis likewise failed to show high assays, in spite of recent fracture and surgical reduction. Patient 7, with a low assay, had primary hyperparathyroidism with hyperplasia and hypertrophy of all four glands, bilateral testicular atrophy due to mumps orchitis, and chronic lymphatic leukemia. Patient 8, with carcinoma of the breast and metastases, excreted less than 3 m.u./24 hrs. Patients 12 and 13 had primary hypothyroidism; the test gave a low but not a negative result in one and a normal result in the other. Patients 16 and 17 had amenorrhea due to FSH lack. In Patient 16, in whom this disorder was probably congenital and was not accompanied by other manifestations of pituitary dysfunction, the result was normal; in Patient 17, in whom the FSH lack was acquired and in whom there was in addition galactorrhea and slight insulin resistance, the test gave high results.

Effect of ACTH injection

Table 8 shows the increase in corticosteroid excretion in 5 of 6 patients who received injections of ACTH. This finding agrees with the increased

TABLE 7. RESULTS IN MISCELLANEOUS CONDITIONS

Patient, MGH unit #	Diagnosis	Sex, Age (yrs.)	Important features	Corticosteroid assay m.u./24 hrs.	
				Positive for	Negative for
1. E.L. 389508	Osteoporosis, cause unknown	F 31	Severe osteoporosis of postmenopausal type in an otherwise healthy young woman. Marked exacerbation during pregnancy.	3 3, 6 3 3 3	6 — — — —
2. D.B. 452389	Osteoporosis, cause unknown	F 24	Severe osteoporosis of postmenopausal type; loss of 6 inches in height; some evidence of increased intestinal motility; no other abnormality found.	3 12	— 48
3. R.E. 601118	Osteoporosis, cause unknown	M 41	Severe osteoporosis; no other abnormality found.	3	24
4. I.C. 623932	Fractured femur; osteoporosis; malnutrition	F 72	Poor nutrition; anemia; osteoporosis; atrophied skin; absent axillary hair; fracture of femur five days before.	3	24
5. E.H. 623157	Fractured femur; osteoporosis; debility	F 78	Fractured femur three weeks before; operative reduction one week before, followed by pyelonephritis with fever.	3	24
6. J.H. 446655	Hypercalcuria	M 36	Hypercalcuria and nephrolithiasis; etiology not discovered; parathyroid exploration neg.	11	—
7. A.A. 391027	Hyperparathyroidism; hypogonadism; lymphatic leukemia	M 39	History of mumps orchitis; sixteen years of nephrolithiosis; operation disclosed enlargement of all parathyroids, of primary type; lymphatic leukemia discovered on routine blood count.	—	3, 24
8. R.H. 615754	Carcinoma of breast	F 50	Widespread metastases from recurrent carcinoma of breast	—	3
9. E.P.	Chronic myeloid leukemia, terminal	F 27	Known to have leukemia for seven years; until recently, good response to x-ray treatment; in terminal state at time of study.	—	24
10. B.D. 109997	Paget's disease	F 54	Extensive involvement of skull and one femur in Paget's disease.	—	24
11. B.L. 466973	Paget's disease	M 45	Involvement of nearly all the skeleton in widespread Paget's disease; receiving stilbestrol.	3	24
12. M.D. 474151	Myxedema	F 69	Classic myxedema; hypertension; postmenopausal osteoporosis; pyelonephritis; cholesterol, 414 mg. per cent; 17-KS 2.5, mg./24 hrs.; insulin tolerance test showed insulin resistance. Last assay made after three weeks of thyroid therapy.	1, 6 —	2 2, 6
13. A.S. 583121	Hypothyroidism	F 59	B.M.R. −40 per cent; PBI., 0.3 r per cent; hypothyroidism, at first thought to be part of panhypopituitarism; FSH pos. for 96 m.u./24 hrs.; insulin tolerance test showed hypoglycemia unresponsiveness; marked improvement on thyroid therapy.	3	24
14. M.C. 598300	Pituitary adenoma, nonfunctioning	F 58	Failing vision for six months; weakness and some coarsening of features for two years; sella turcica enlarged; serum P, 2.5 mg. per cent; FSH neg. for 6.5 m.u./24 hrs.; 18-KS, 2.3 mg./24 hrs.; insulin tolerance test showed slight insulin resistance.	—	3, 24
15. A.G. 469138	Hypertension	M 53	B.P. 210/120; receiving low sodium diet.	3	24
16. C.W. 229892	Primary FSH lack	F 18	Primary amenorrhea; no breast development; pubic hair at 16 yrs.; growth and epiphyseal development normal; FSH neg. for 3 m.u./24 hrs.; 11-OCS, .18 to .09 mg./24 hrs.; insulin tolerance test normal.	3	?24
17. I.R.	Pituitary amenorrhea	F 37	Amenorrhea 7 yrs.; slight hirsutism; galactorrhea; FSH pos. for 6.5 m.u./24 hrs.; neg. for 13 m.u./24 hrs.; insulin tolerance test shows slight insulin resistance.	3, 24	—
18. F.M. 458093	Calcification of adrenals without adrenal insufficiency	F 33	Symptoms at first thought to be due to Addison's disease disappeared after cholecystectomy. Insulin tolerance test did not show hypoglycemia unresponsiveness.	3	—
19. J.L. 591046	Anorexia nervosa	F 20	Classic anorexia nervosa; wt. loss from 130 to 85 lbs.; amenorrhea past 5 mos.	3, ? 24	—
20. F.H. 569024	Anorexia nervosa	F 21	Unhappy past 4 yrs.; amenorrhea 3½ yrs.; wt. loss past 2 yrs. from 110 to 80 lb.; FSH neg. for 3 m.u./24 hrs.; 17-KS 4.7 mg./24 hrs.; insulin tolerance test shows slow fall and slow rise of blood sugar.	3	24

TABLE 8. RESULTS OF ACTH INJECTION

Patient	Diagnosis	Corticosteroid assay before admin. of ACTH m.u./24 hrs.		Corticosteroid assay during admin. of ACTH m.u./24 hrs.		Days ACTH administered	Corticosteroid assay after cessation of ACTH m.u./24 hrs.		Days after cessation of ACTH	Amount of ACTH used (mg. of standard)
		Positive for	Negative for	Positive for	Negative for		Positive for	Negative for		
1. R.H., Case #6, Table 3	Metastatic carcinoma of breast		3	24		3rd to 6th day	3	24	14	72 mg. daily for 18 days followed by 96 mg. for 12 days
			—	24		11 days				
2. C.O., Case #1, Table 6	Adrenogenital syndrome		3		4	1st—5 days	3	24	1 to 8	37 mg. daily for 12 days
			2.8		3	2nd—8 days				
			2.8							
3. M.H., Case #2, Table 5	Panhypopituitarism		2.8	2.8		1		2.8	1 to 6	43 mg. daily for 7 days
			2.8							
			2.8							
4. H.J., Case #3, Table 5	Panhypopituitarism		1.5	1.5		1 to 6		1.5	1 to 6	45 mg. daily for 19 days.
			1.5	—	1.5	6–12				
			1.5	—	1.5	12–18				
			1.5							
4. H.J., Case #3, Table 5	Panhypopituitarism		1.5	3	24	1– 4		3	1–5	43 mg. daily for 13 days.
			1.5	3		4– 8				
			1.5	3.75		8–12				
			1.5							
5. B.D., Case #10, Table 7	Paget's disease		24	24		1 and 2				73 mg. daily for 3 days.
6. F.P., Case #7, Table 7	Terminal myeloid leukemia		24	24		2				63 mg. daily for 2 days.

corticosteroid excretion known to follow damaging events (16). The sixth patient, who did not respond, had the adrenogenital syndrome (discussed previously).

SUMMARY AND CONCLUSIONS

1. A modification of Eggleston and Dobriner's method for biologic assay of urinary corticosteroid is described. Results are expressed in mouse units per 24 hours.

2. Of 32 assays on 25 normal young adults of both sexes, all except one were positive for 3 m.u./24 hrs. and all except two were negative for 24 m.u./24 hrs.

3. Of 9 cases of Addison's disease, in none was there any detectable urinary corticosteroid.

4. Of 8 classic cases of Cushing's syndrome, in none was the excretion less than 24 m.u./24 hrs.

5. Of assays in 8 cases of acromegaly, 1 was positive for 24 m.u./24 hrs.; 2 were negative for 3 m.u./24 hrs., and 6 were within normal range.

6. Of 8 classic cases of panhypopituitarism, in none was there any detectable urinary corticosteroid; in 5 additional cases of less complete hypopituitarism, the test gave negative results for 3 m.u./24 hrs. in all except 1.

7. Of 4 female patients with the adrenogenital syndrome, 3 were tested for 3 m.u./24 hrs. and gave a negative response; 1 was tested for 24 m.u./24 hrs. and also gave a negative response.

8. Miscellaneous observations are included on cases of idiopathic osteoporosis, senile osteoporosis, Paget's disease, idiopathic hypercalcuria, malignancy, amenorrhea due to FSH lack, hyperparathyroidism, primary hypothyroidism, anorexia nervosa, and hypertension.

9. Increase of corticosteroid excretion was obtained in 5 out of 6 patients given ACTH injections; the 1 patient who did not respond had the adrenogenital syndrome.

10. In general, there is rough correlation between the results of this test and the results with chemical methods; but in certain conditions (Addison's disease, hypopituitarism) a low excretion is better shown by the biologic test. Moreover, if our findings in the adrenogenital syndrome can be repeated, in this condition the tests actually diverge.

11. The authors agree with Venning, Ripstein and Kazmin (16) that the biologic test cannot be completely superseded by the chemical methods. They have no conviction that the biologic method here presented is superior to the two others mentioned. Although cumbersome and in need of simplification, it appears to be reliable.

REFERENCES

1. Reinecke, R. M., and Kendall, E. C.: Method for bio-assay of hormones of adrenal cortex which influence deposition of glycogen in the liver, *Endocrinology* **31**: 537, 1942.
2. Eggleston, N. M.: Johnston, B. J., and Dobriner, K.: Quantitative methods for the bio-assay of the glycogenic acitivity of steroids and urinary extracts, *Endocrinology* **38**: 197, 1946.
3. Venning, E. H.; Kazmin, V. E., and Bell, J. C.: Biological assay of adrenal corticoids, *Endocrinology* **38**: 79, 1946.
4. Dorfman, I.; Ross, E., and Shipley, R. A.: The assay of adrenal cortical material by means of a glycogen test in the adrenalectomized mouse, *Endocrinology* **38**: 178, 1946.
5. Venning and Kazmin; Dobriner, Lieberman and Eggleston; Griswold and Forbes; Dorfman, Shipley, Schiller and Ross: Symposium on urinary corticosteroids, *Tr. Josiah Macy, Jr. Foundation Conferences*, Conference on Metabolic Aspects of Convalescence Including Bone and Wound Healing, Tenth Meeting, June 15–16, 1945, p. 131.
6. Agate, F. J., Jr., and Zwemer, R. L.: Some factors affecting adrenal insufficiency in the rat, *Am. J. Physiol.* **111**: 1, 1935.
7. Nelson, N.: A photometric adaptation of the Somogyi method for the determination of glucose, *J. Biol. Chem.* **153**: 375, 1944.

8. LIEBERMAN, S.; HARITON, L. B., and FUKUSHIMA, D. K.: Studies in steroid metabolism; the problem of Walden inversion in the reactions of steroid hydrogen sulfates and steroid sulfites, *J. Am. Chem. Soc.* **70:** 1427–32, 1948.
9. ENGEL, LOUIS: Personal communication.
10. HEARD, R. D. H.; SOBEL, H., and VENNING, E. H.: The neutral lipide-soluble reducing substances of urine as an index of adrenal cortical function, *J. Biol. Chem.* **165:** 699, 1946.
11. TALBOT, N. B.: Personal communication.
12. TALBOT, N. B.; SALTZMAN, A. H.; WIXOM, R. L., and WOLFE, J. K.: The colorimetric assay of urinary corticosteroid-like substances, *J. Biol. Chem.* **160:** 535, 1945.
13. ALBRIGHT, F., and ELRICK, H.: An attempt to classify hormonal disorders of the hypophysis, *Tr. A. Am. Physicians* **61:** 42, 1948.
14. FISHMAN, J. B.; WILHELMI, A. E., and RUSSELL, J. A.: Preparation of crystalline growth hormone, (abstract) *J. Clin Endocrinol.* **8:** 593, 1948.
15. TALBOT, N. B.; ALBRIGHT, F.; SALTZMAN, A. H.; ZYGMUNTOWICZ, A. and WIXOM, R.: The excretion of 11-oxycorticosteroid-like substances by normal and abnormal subjects, *J. Clin. Endocrinol.* **7:** 331, 1947.
16. WEIL, P. G., and BROWNE, J. S. L.: Excretion of cortin after surgical operation, *Science* **90:** 445, 1939.
17. VENNING, E. H.; RIPSTEIN, M. P., and KAZMIN, V. E.: Urinary corticoids, (abstract) *J. Clin. Endocrinol.* **9:** 659, 1949.

EFFICACY OF TESTOSTERONE COMPOUNDS ADMINISTERED SUBLINGUALLY TO HYPOGONADAL MEN*

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OBJECTIVE evidence of the effectiveness of methyltestosterone administered by the sublingual or buccal route has been previously reported by Joël in 1942; Spence, 1942; Lisser, Escamilla, and Curtis, 1942; Lisser and Curtis, 1943 and 1945; Dunn and Hoffman, 1945; Finkler, 1947; Deamer, 1948; and Escamilla, 1949 (1–9). This method of administration is not new, as nitroglycerine has been given by this route for many years. The pharmacology of the method was discussed by Walton in 1944 (10). Sublingual administration of steroidal hormones was first shown to be effective by Anderson, Haymaker, and Henderson (11), who used desoxycorticosterone acetate dissolved in propylene glycol. A comparison of testosterone and its esters administered in propylene glycol was reported from this clinic in 1942 (3). However, the use of the propylene glycol solvent was not popular with the patients. They reported an unpleasant taste, associated salivation, and objected to the inconvenience of holding the tongue in a retracted position for several minutes. It was difficult to be certain of the exact dose of steroid in this solvent because of the variable size of the drops and the varying solubility of the steroid at different temperatures. The use of compressed tablets, of sufficient hardness to dissolve at a suitable rate, was found to circumvent all of these difficulties, and, as already noted, the efficacy of methyltestosterone given in this manner has been well documented.

It is the purpose of this report, first, to compare the clinical effectiveness of methyltestosterone, unesterified testosterone, and testosterone propionate tablets in a lactose base[1] and, second, to compare with these, unesteri-

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[1] Grateful acknowledgment is made to Dr. E. Oppenheimer, Ciba Pharmaceutical Products, Inc., Summit, New Jersey, for generous quantities of hard compressed tablets of methyl testosterone (Metandren Linguets), unesterified testosterone, testosterone propionate, and placebos in a similar lactose base.

fied testosterone in a gelatin-sucrose base,[2] as well as testosterone propionate and unesterified testosterone in a carbowax base.[3]

METHOD

The preparations were assayed in 12 hypogonadal male patients in the University of California Out-Patient Department. This type of individual responds specifically and in a dramatic manner to androgenic therapy and therefore represents the ideal test object for effectiveness of an androgenic preparation. Since this is substitution therapy and regression occurs it if is not maintained, various preparations can be compared serially in the same patient.

The group of 12 patients consisted of 2 eunuchs (Cases 2 and 6), 3 eunuchoids with high gonadotropin excretion (Cases 4, 5, and 7), 2 eunuchoids with normal gonadotropin excretion (Cases 3 and 10), and 5 eunuchoids with low or absent gonadotropin excretion (Cases 1, 8, 9, 11 and 12). In the last group, Case 1 was that of a patient with hypogonadotropic eunuchoidism associated with an intrasellar tumor.

The initial androgenic therapy in 7 of these patients was administered sublingually or buccally. Since the initial response is always the most dramatic, results in these cases were particularly convincing. The other 5 patients had been treated previously with parenteral androgens, but in most instances treatment had lapsed before the start of buccal or sublingual therapy.

Results were classified by an arbitrary system in which the number of plus signs denoted the degree of androgenic effect. A *full androgenic effect* was recorded as three plus (+++). Results were so classified if the patient had growth of the genitalia to normal size; one to three erections per day; increase in facial, pubic and axillary hair; normal capacity for intercourse, masturbation, or seminal emissions; deepening of the voice; acne; and such general metabolic effects as initial increase and subsequent maintenance of weight and strength.

In some instances an *excessive effect* occurred, characterized principally by priapism; four or more erections per day; and, occasionally, edema. This was taken as an indication of overdosage and recorded as four plus (++++).

A *moderate androgenic effect* (three to seven erections per week) was indicated by two plus (++). A *slight androgenic effect* (usually associated with less than three erections per week) was recorded as one plus (+).

[2] Testosterone Lingusorbs, kindly furnished by Dr. J. Murray Scott, Ayerst, McKenna and Harrison, Ltd., New York.

[3] Buccal tablets in Polyhydrol base generously furnished by Dr. Edward Henderson, Schering Corporation, Bloomfield, New Jersey.

Although the entire clinical picture was considered, the number of erections was found to be the most reliable single indicator of the androgenic response in these patients. The sexual function, as measured by the ability to have intercourse and ejaculation, was usually proportionate. A doubtful effect was recorded as plus or minus (±), and complete absence of response was recorded as zero (0).

The agents used were all administered sublingually or buccally in hard compressed tablets of various bases. One of these, into which was incorporated either methyltestosterone, testosterone, testosterone propionate, or inert material (placebo), contained lactose, granulated sugar, magnesium stearate, talc, and tragacanth. This is the preparation (linguet) which has been used in this clinic for seven years and in which sublingual methyltestosterone has been shown to have full androgenic effects. More recently a waxy base of polyethylene glycol has been made available containing either unesterified testosterone or testosterone propionate. In this tablet the steroid is dissolved in a polyethylene glycol wax. The last base available for this study was composed of powdered gelatin and powdered sucrose with a small amount of stearic acid and acacia as binders. It contained only unesterified testosterone.

When the sublingual route was used, the tablets were placed under the tongue and allowed to dissolve slowly while the subject refrained from eating, drinking, talking, smoking and moving the tongue about. Since this mode of administration interfered with talking, some patients preferred placing the tablets in the buccal gutter (vestibulum oris) between the lower gum and the cheek. In this position, the tablets did not interfere with tongue movements but took longer to dissolve.

CASE REPORTS[4]

Case 1. L. D. (U128096): Eunuchoidism secondary to lack of pituitary gonadotropins associated with a tumor of the pituitary gland

This patient (Fig. 1) was 34 years of age and complained that he had not matured. At the age of 17 or 18 he had experienced frequent headaches with mild nausea but these had subsided. Examination showed him to be slender with eunuchoid proportions. His height was 68 inches, symphysis to floor 38 inches and span 71 inches. His complexion was fawn-colored and his face had a slightly progeric cast. Pubic and axillary hair were absent and his face was smooth. The genitalia were infantile. Ocular fundi were normal, as were the visual fields. The BMR was −18 per cent. A five-hour glucose tolerance curve

[4] The case reports were originally prepared in considerably more detail in order to document fully the effects reported. However, at the request of the editorial board the brief abstracts presented here have been substituted. Photographs are available of the patients, but only a few showing representative effects have been chosen for publication in order to conserve space. If further details concerning any of the cases are desired the authors will be glad to supply them.

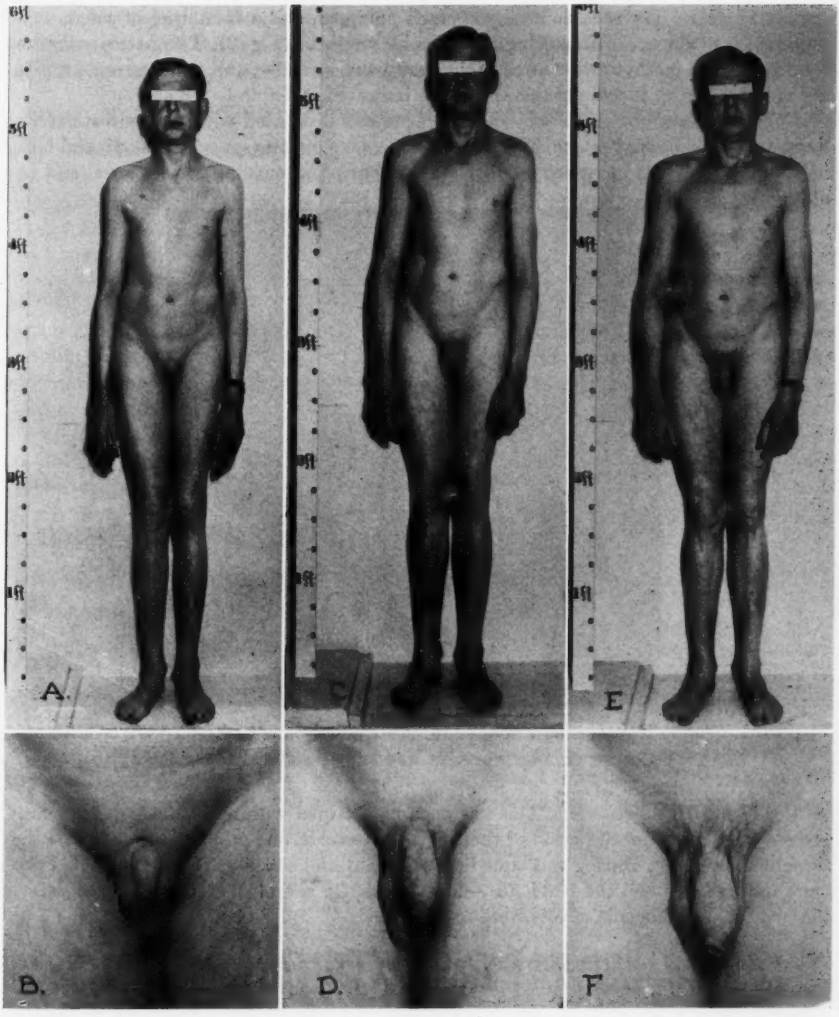

FIG. 1. *Case 1.* Eunuchoidism secondary to lack of pituitary gonadotropins associated with a tumor of the pituitary gland.

A and B: 5/6/46. Appearance before treatment.

C and D: 10/17/46. After five months of treatment with 20 mg. daily of methyltestosterone in lactose base sublingually. Note genital and muscular development.

E and F: 5/22/47. After one year of sublingual therapy. Note further development and appearance of pubic hair.

was normal and urinary 17-ketosteroid excretion (ketonic fraction) was 3.8 mg. per 24 hours. The FSH assay was negative at 3 mouse uterine units. The bone age was approxi-

mately 17 years. The sella turcica was much enlarged, and a faint ring of calcification was seen just above it, suggesting a craniopharyngioma (Fig. 2). The patient received treatment with methyltestosterone sublingually and buccally, and testosterone propionate in lactose base. Results of therapy are as indicated in Table 1.

Effects of therapy: Satisfactory development was instituted with methyltestosterone linguets, 20 mg. per day, alone. There was phallic development, growth of body and facial hair, gain in weight, increased muscular development, deepening of the voice, and rise

FIG. 2. *Case 1.* Roentgenogram of skull. Note enlargement of sella with erosion of posterior clinoid processes and suprasellar calcification.

of the BMR to −9 per cent. This has been maintained with one 10-mg. linguet daily, except for a four-month period of therapy with testosterone propionate tablets (lactose base) sublingually. During this time he lost weight and sexual capacity, the blood pressure fell to 85/55 and the BMR to −26 per cent. On resumption of methyltestosterone linguets, full androgenic effects were re-instituted.

Case 2. W. O. (U135482): Eunuchism secondary to bilateral intrascrotal abscesses

This patient was seen at the age of 38, six months after the occurrence of bilateral intrascrotal abscesses. Following this, libido and potentia had disappeared, pubic and axillary hair had thinned and there was decreased need for shaving. He had noticed hot flushes and increased nervousness, irritability and inability to concentrate. Examination revealed a fawn-colored complexion with some wrinkling about the eyes. Pubic and axillary hair were scarce and the trochanteric and mammary fat pads were prominent. Scrotal contents were palpable only as small nubbins of tissue. 17-Ketosteroid excretion (ketonic fraction) was 11.5 mg. per day and the FSH assay was positive at 192 mouse uterine units per day. The patient received methyltestosterone and testosterone propionate in lactose base. The results were as indicated in the table.

Effects of therapy: Methyltestosterone linguets, in a dose of 15 mg. per day, restored sexual activity and abolished climacteric symptoms in a 38-year-old man who lost testicular function following bilateral intrascrotal abscesses.

Case 3. D. C. (U137918): Hypogonadism with normal FSH (Fröhlich type)

This patient was seen at the age of 20 and complained that he had never matured. His testes had not descended until after some injection therapy at the age of 18. He had gained 25 pounds in the two years preceding examination here, with deposition of fat mostly on the trunk and some girdle accentuation. Examination showed the hair line to be low on the temples with fine wrinkling about the eyes and mouth. There was also a generalized ichthyosis. Axillary and pubic hair were sparse and there was no beard growth. The genitalia were infantile and the testes migratory. The BMR was −4 per cent and the bone age was approximately 15 years. 17-Ketosteroid excretion (ketonic fraction) was 8.1 mg. FSH assay was positive at 6 and negative at 51 mouse uterine units per day. This patient received methyltestosterone in lactose base and unesterified testosterone in the gelatin-sucrose base. Results of therapy with various doses of each are shown in the table.

Effects of therapy: Genital maturation was induced in a 20-year-old hypogonadal man, with 20 mg. of methyltestosterone linguets per day. Maintenance was accomplished with 10 mg. of methyltestosterone sublingually per day or 12 mg. of unesterified testosterone. sublingually per day.

Case 4. K. B. (U141372): Preadolescent eunuchoidism with high FSH (primary testicular eunuchoidism)

The patient reported that undescended testes and sexual retardation were first noted at the age of 14. He had continued to grow in height beyond the usual age, reaching 77 inches at the age of 23. Previous treatment had been given at the age of 24 by Dr. Cyril MacBryde at the Barnes Hospital in St. Louis. This had included chorionic gonadotropin and later, intramuscular testosterone propionate. Treatment had lapsed for three and one-half years when he was seen here at the age of 32. He complained of hot flushes and inability to concentrate, as well as diminished sexual function. On examination the patient was tall with typical eunuchoid proportions. Facial and axillary hair were scant and the pubic hair was of feminine distribution. Genitalia were small and the left testis could not be palpated. The BMR was −26 per cent and the bone age was approximately 17 years. 17-Ketosteroid excretion (ketonic fraction) was 8.5 mg. per 24 hours. The test for FSH in the urine gave a positive result at 96 mouse uterine units. This patient received methyltestosterone in lactose base with good results as indicated in the table. At one time oral methyltestosterone tablets, designed to be swallowed, were dispensed by error and the patient noted that they dissolved within five minutes. During three months of this therapy there was a definite loss of effect. This was corrected by resuming the use of linguets in the same dose.

Effects of therapy: Sexual activity, weight gain and abolition of climacteric symptoms were produced in a 32-year-old man with preadolescent eunuchoidism, following therapy with 10 mg. per day of methyltestosterone in hard compressed linguets. Ordinary tablets in the same amount placed in the sublingual position were ineffective.

Case 5. M. F. (U126383): Preadolescent eunuchoidism with normal FSH

This patient was 32 years of age and gave a history of failure to mature sexually. He had continued to grow in height until the age of 29. Previous therapy with intra-

muscular injections had resulted in considerable improvement in his sexual status. This treatment had lapsed for three years when he was seen here. Examination showed him to be tall with disproportionately long arms and legs. The mammary and trochanteric fat pads were prominent and there was some scoliosis. The genitalia were small. 17-Ketosteroid excretion (ketonic fraction) was 13.3 mg. per day and FSH assay was positive at 5 and negative at 80 mouse uterine units. The bone age was adult but revealed osteoporosis and epiphysitis of the thoracolumbar spine. The patient received methyltestosterone in lactose base with results as recorded in the table.

Effect of therapy: Ten milligrams of methyltestosterone sublingually was found to be a full maintenance dose in this 32-year-old man with preadolescent eunuchoidism.

Case 6. A. R. (U85578), (Case 11 in a previous paper (4)): Eunuchism

This patient was seen in 1942 at the age of 73 and complained of melancholia, hot flushes and nervousness since castration following testicular injury one year before. He also had hypertensive cardiovascular disease with congestive failure, and laboratory evidence of meningovascular syphilis. After some intramuscular injections of testosterone propionate, therapy was changed to methyltestosterone in lactose base administered sublingually. This resulted in considerable general improvement as indicated in the table. 17-Ketosteroid excretion (total neutral steroids) was 5.1 mg. per 24 hours. FSH assay was positive at 5 and negative at 80 mouse uterine units.

Effects of therapy: A 73-year-old man, who developed severe climacteric symptoms following castration, was fully maintained free of symptoms with restoration of sexual activity, for six years, during therapy with one 5-mg. methyltestosterone linguet daily.

Case 7. L. V. (U89575), (Case 9 in a previous paper (4)): Preadolescent eunuchoidism with high FSH (primary testicular eunuchoidism)

This patient was seen at the age of 19 and was an instance of typical preadolescent eunuchoidism. He took methyltestosterone linguets for five and one-half years with good response. On examination at age 25 he had eunuchoid proportions. The facial hair was scant; axillary and pubic hair were moderately abundant. The penis was of normal size but no testes could be identified. 17-Ketosteroid excretion (total neutral) was 13.7 mg. and the FSH assay was positive at 80 mouse uterine units. Chorionic gonadotropin (APL)[5] was administered for two months in order to see if testicular descent could be attained. It was unsuccessful and the patient has subsequently been maintained on methyltestosterone in lactose base.

Effects of therapy: A 19-year-old youth with preadolescent eunuchoidism has been fully maintained for five and one-half years with one 10-mg. methyltestosterone linguet per day. Chorionic gonadotropin, 1000 I.U. three times weekly, was less effective.

Case 8. J. J. (U36521), (Case 4 in previous papers (3, 12, 13)): Preadolescent eunuchoidism with low FSH

This patient was 31 years old when first seen in 1938. He was an instance of typical untreated preadolescent eunuchoidism. 17-Ketosteroid excretion was 1.4 mg. per 24 hours and the FSH assay was negative at 5 mouse uterine units. He showed the characteristic effects, over a five-year period, of treatment with testosterone propionate

[5] Furnished by Dr. J. Murray Scott, Ayerst, McKenna and Harrison, Ltd., New York.

administered intramuscularly, implants of methyltestosterone, methyltestosterone swallowed, and various testosterone compounds administered sublingually in propylene glycol. This patient had come to recognize the effects of testosterone therapy and was a good subject for comparing the various types and modes of administration. For the present study he received methyltestosterone in lactose base, testosterone propionate in lactose base, placebos in lactose base, unesterified testosterone in lactose base, unesterified testosterone in gelatin-sucrose base, and testosterone propionate in the carbowax base. Comparative effects of the therapy are recorded in the table.

Effects of therapy: A 31-year-old man with preadolescent eunuchoidism was fully maintained with one 10-mg. methyltestosterone linguet per day. Twice as much unesterified testosterone was required to produce the same effect. Forty milligrams of testosterone propionate in carbowax failed to produce an equivalent response.

Case 9. J. F. (U80642). (Case 10 in a previous paper (4)): Hypogonadism associated with hypopituitarism

This patient was 43 years old when first seen in 1942. He complained of short stature and lack of sexual maturity. Examination showed him to be $58\frac{1}{4}$ inches in height with disproportionately long arms and legs. The skin was dry and finely wrinkled. There was no beard, axillary hair was sparse and pubic hair was absent. The genitalia were infantile. The BMR was −12 per cent and the bone age was 16. 17-Ketosteroid excretion (ketonic fraction) was 5.9 mg. per 24 hours. FSH assay was negative at 6.4 mouse uterine units. The first type of treatment was with methyltestosterone as the swallowed tablet. This was compared with methyltestosterone administered sublingually in lactose base. Later the patient served as a test subject for various testosterone compounds administered sublingually in propylene glycol. For the present study, the patient received methyltestosterone in lactose base, testosterone propionate in lactose base and unesterified testosterone in lactose base. Results are reported in the table.

Effects of therapy: A 43-year-old man with hypogonadism associated with hypopituitarism was found to have a full androgenic response during therapy with 10 mg. of methyltestosterone or unesterified testosterone buccally per day, but received less effect from the same dose of testosterone propionate.

Case 10. H. R. (U126236), (reported previously (9)): Preadolescent eunuchoidism with normal FSH

This patient was seen at the age of 19 and complained of "lack of development." He had continued to grow in height beyond the usual age. On examination he was tall with eunuchoid proportions and appeared immature. Facial, axillary and pubic hair were scant. The genitalia were tiny. Bone age was 17 to 18 years. 17-Ketosteroid excretion was 9.5 mg. per 24 hours (total neutral). FSH assay was positive at 6 and negative at 18 mouse uterine units. Treatment included methyltestosterone in lactose base, testosterone propionate in lactose base, testosterone propionate in carbowax base, unesterified testosterone in lactose base and unesterified testosterone in gelatin-sucrose base. The comparative results are recorded in the table.

Effects of therapy: A 19-year-old youth with preadolescent eunuchoidism was fully maintained on 5 mg. of methyltestosterone sublingually per day, but was not fully maintained on four times that amount of unesterified testosterone. Sublingual testosterone propionate was relatively inert in this patient.

Case 11. G. W. (U142101): Preadolescent eunuchoidism with low FSH ("familial hypogonadotropic eunuchoidism")

This patient (Fig. 3) was seen at the age of 17 because of right unilateral cryptorchidism associated with eunuchoidism. He had received previous treatment with small doses of chorionic gonadotropin. Two brothers were similar to the patient. Examination revealed eunuchoid proportions, sparse axillary and pubic hair and small genitalia. Bone age was 12 to 13 years. The right testis was brought down surgically. The patient then

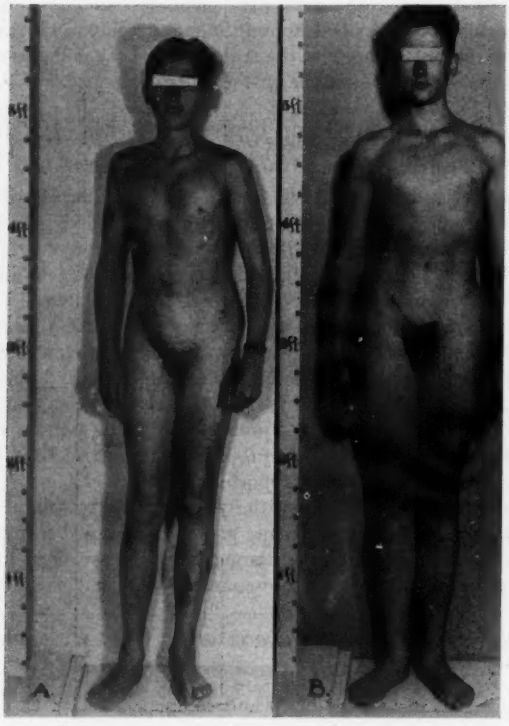

FIG. 3. *Case 11.* Preadolescent eunuchoidism with low FSH.

A: 7/1/47. Appearance after Torek operation and before androgenic therapy.

B: 9/29/48. Appearance after nine months of ineffective treatment and four months of treatment with methyltestosterone, 20 mg. in lactose sublingually per day. Note genital and somatic development.

had the following therapy serially: placebo in lactose base, testosterone propionate in lactose base, testosterone propionate in carbowax base, unesterified testosterone in lactose base, and unesterified testosterone in carbowax base. All of the above were ineffective in initiating any androgenic response in doses as great as 60 mg. per day. Methyltestosterone in lactose base was then started with good clinical improvement. Later, the effect of unesterified testosterone in gelatin-sucrose base was compared as recorded in the table.

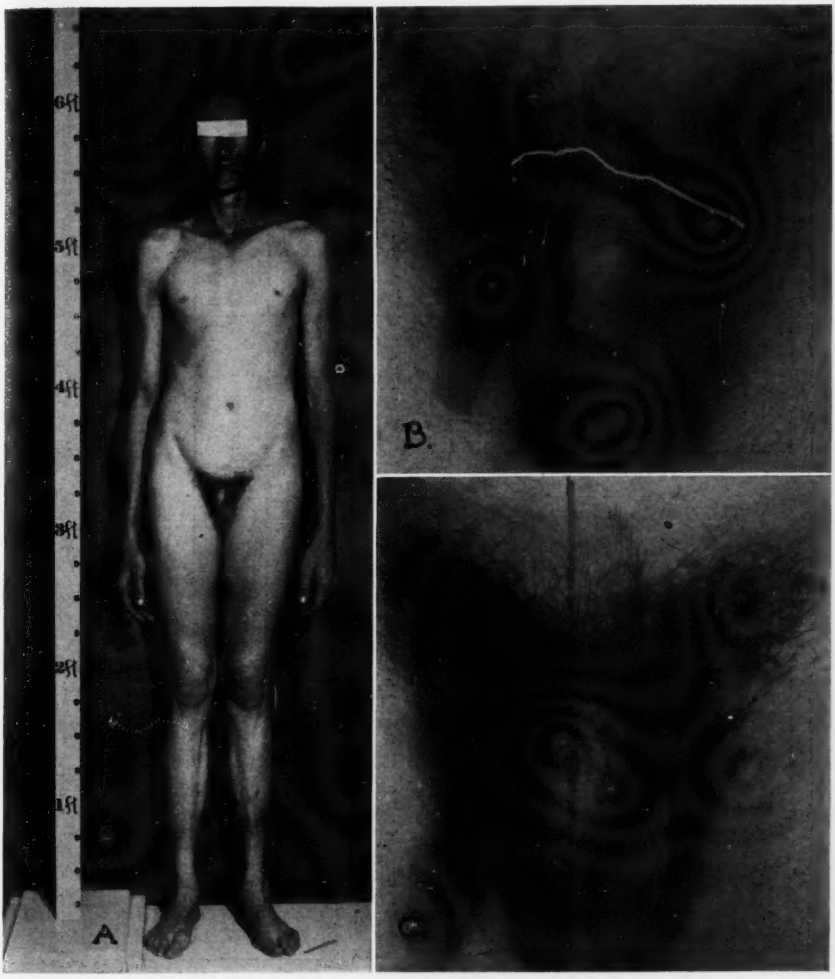

FIG. 4. *Case 12.* Preadolescent eunuchoidism with low FSH.
A and B: 12/6/48. Patient before therapy.
C: 2/2/49. Genitalia after six weeks of treatment with unesterified testosterone in gelatin-sucrose base, 48 mg. per day buccally. Note enlargement of phallus.

Effects of therapy: A 17-year-old youth with preadolescent eunuchoidism failed to show an initial androgenic response to unesterified testosterone or testosterone propionate sublingually in doses as large as 60 mg. per day in lactose or carbowax bases. During therapy with 20 mg. of methyltestosterone sublingually per day a full effect was initiated and was subsequently maintained on 10 mg. of methyltestosterone sublingually per day.

TABLE 1. ANDROGENIC EFFECTS PRODUCED IN HYPOGONADAL MALES BY VARIOUS TESTOSTERONE COMPOUNDS ADMINISTERED AS SUBLINGUAL OR BUCCAL TABLETS

(Daily dose, androgenic effect, and length of observation period)

Case	Methyltestosterone in lactose base	Unesterified testosterone in lactose (L), gelatin-sucrose (G), or wax (W) base	Testosterone propionate in lactose (L) or carbowax (W) base	Remarks
1. (L.D.) Age 34–38	*20 mg. +++ (14 mo.) 15 mg. +++ (6 mo.) 10 mg. +++ (6½ mo.) (10 mo.)		20 mg. L + (2 mo.) 10 mg. L ± (2 mo.)	*Hypogonadotropic eunuchoidism with pituitary tumor.* Status at start of sublingual therapy: 0 (androgenic status). Sublingual and buccal administration of methyltestosterone linguets produced equivalent effect.
2. (W.O.) Age 38–41	*20 mg. +++ (4 mo.) 15 mg. +++ (10 mo.) (12 mo.)		30 mg. L + (1 mo.) 15 mg. L ± (2 mo.)	*Postinfectious eunuchism.* Status at start of sublingual therapy: 0
3. (D.C.) Age 20–22	*20 mg. +++ (5 mo.) 40 mg. ++++ (1 mo.) 15 mg. +++ (9 mo.) 10 mg. +++ (7 mo.) (1 mo.)	18 mg. G +++ (1 mo.) 12 mg. G +++ (1 mo.)		*Hypogonadism with normal FSH.* Status at start of sublingual therapy: 0
4. (K.B.) Age 32–34	10 mg. +++ (4 mo.) (4 mo.) 10 mg. *oral tablets* + (3 mo.)			*Primary testicular preadolescent eunuchoidism.* Previously treated with parenteral testosterone propionate. No therapy for 3½ years. Status at start of sublingual therapy: +
5. (M.F.) Age 32–35	20 mg. ++++ (2 wk.) 15 mg. ++++ (1 mo.) 10 mg. +++ (4 mo.) (27 mo.)			*Preadolescent eunuchoidism with normal FSH.* Previously treated with parenteral testosterone propionate. No therapy for 3 years. Status at start of sublingual therapy: 0
6. (A.R.) Age 73–79	10 mg. +++ (72 mo.) 5 mg. ++ (4 mo.)			*Eunuchism.* Previously treated with testosterone propionate parenterally and sublingually in propylene glycol.
7. (L.V.) Age 19–25	*15 mg. +++ (5 mo.) 20 mg. +++ (2 mo.) 10 mg. +++ (63 mo.)			*Primary testicular preadolescent eunuchoidism.* Status at start of sublingual therapy: 0. Therapy omitted for 1 mo., Nov.–Dec. 1946: effect dropped to 0. APL 1,000 IU 3 times weekly: ++
8. (J.J.) Age 35–41	20 mg. ++++ (1 mo.) 15 mg. +++ (1 mo.) 10 mg. +++ (6 mo.) (4 mo.) 5 mg. +++ (1 mo.) (23 mo.) 2½ mg. ++ (1 mo.) (12 mo.) (2 mo.) 1.7 mg. + (6 mo.)	20 mg. L +++ (1 mo.) (1 mo.) 10 mg. L ++ (1 mo.) 10 mg. G ++ (1 mo.)	40 mg. W ++ (1 mo.) 20 mg. L ++ (1 mo.) 20 mg. W ++ (1 mo.) 15 mg. L ++ (3 mo.) 10 mg. W ++ (1 mo.) 5 mg. L + (2 mo.)	*Hypogonadotropic eunuchoidism.* Previously treated with several testosterone preparations and gonadotropins. Therapy omitted for 17 mo., Nov. 1942–March 1944: 0. Placebo linguets: 0. Sublingual and buccal administration of methyltestosterone linguets produced equivalent effect.

9. (J.F.) Age 43–49	25 mg ++++ (1 mo) 20 mg. +++/++++ (1 mo.) 15 mg. +++ (2 mo.) (4 mo.) 10 mg. +++ (1 mo.) (2 mo.) 5 mg. ++ (6 mo.)	20 mg. L +++ (1 mo.) 10 mg. L +++ (2 mo.)	10 mg. L ++ (1 mo.) 5 mg. L +/++ (1 mo.)	*Hypogonadism associated with hypopituitarism.* Previously treated with methyltestosterone orally and various testosterone compounds sublingually in propylene glycol.
10 (H.R.) Age 19–22	*20 mg ++++ (9 da) (10 da.) 10 mg. ++++ (7 mo.) (2 mo.) 10 mg. +++ (3 wk.) 5 mg. +++ (5 mo.) (1 mo.) 5 mg. ++ (10 mo.)	20 mg. L ++ (5 mo.) 10 mg. G ++ (1 mo.) 10 mg. L + (1 mo.) 6 mg. G ++ (1 mo.)	20 mg. W + (1 mo.) 10 mg. L + (1 mo.) (1 mo.) 10 mg. W + (1 mo.) (1 mo.) 5 mg. L ± (2 mo.) (1 mo.)	*Preadolescent eunuchoidism with normal FSH.* Status at start of sublingual therapy: ±
11. (G.W.) Age 17–19	*20 mg. +++ (11 mo.) 10 mg. +++ (1 mo.)	*30 mg. L 0 (1 mo.) *30 mg. W 0 (1 mo.) 18 mg G ++ (1 mo)	*60 mg. W 0 (1 mo.) *30 mg. W 0 (1 mo.) *30 mg L 0 (1 mo.) *20 mg L 0 (1 mo.) *10 mg. L 0 (1 mo.)	*Familial hypogonadotropic eunuchoidism.* Status at start of sublingual therapy: 0. Placebo linguets: 0
12. (C.W.) Age 25	10 mg. +++ (1 mo.)	*24 mg. G 0 (1 wk.) *48 mg. G +++ (6 wk.) 40 mg. L +++ (1 mo.) 20 mg. L +++ (1 mo.)	20 mg. W 0 (1 mo.)	*Familiar hypogonadotropic eunuchoidism.* Status at start of buccal therapy: ±
Summary	40 mg. +++½ (1 case) 25 mg. ++++ (1 case) 20 mg. +++ to ++++ (9 cases) 15 mg. +++ to ++++ (6 cases) 10 mg. +++ to ++++ (11 cases) 5 mg. ++ to +++ (4 cases) 2½ mg. ++ (1 case) 1.7 mg. + (1 case)	48 mg. +++ (G, 1 case) 40 mg. +++ (L, 1 case) 30 mg. 0 (L/W, 1 case) 20 mg. ++ to +++ (L, 4 cases) 18 mg. ++ to +++ (G, 2 cases) 12 mg. ++ to +++ (G, 2 cases) 10 mg + to +++ (L, 3 cases) (G, 2 cases) 6 mg ++ (G, 1 case)	60 mg. 0 (W, 1 case) 30 mg. 0 to + (L, 2 cases) (W, 1 case) 20 mg. 0 to ++ (L, 3 cases) (W, 1 case) 15 mg. ± to ++ (L, 2 cases) 10 mg. 0 to ++ (L, 4 cases) (W, 1 case) 5 mg. ± to +/++ (L, 3 cases)	

* Initial androgenic therapy.

Androgenic effect: 0 None; ± Doubtful; + Slight; ++ Moderate; +++ Full; ++++ Excessive.

Case 12. C. W. (U160776): Preadolescent eunuchoidism with low FSH ("familial hypogonadotropic eunuchoidism")

This patient (Fig. 4), age 25, is the brother of G. W. (Case 11). He also complained of retarded genital development and an undescended right testis. On examination he had disproportionately long arms and legs. Facial hair was absent and there was feminine distribution of pubic hair. Genitalia were small. The BMR was −54 per cent on two occasions. 17-Ketosteroid excretion (total neutral) was 10.6 mg. per 24 hours. FSH assay was negative at 5 mouse uterine units. The sella turcica was normal and the bone age was between 16 and 18 years. Therapy was started with unesterified testosterone in gelatin-sucrose base, testosterone propionate in carbowax base and methyltestosterone in lactose base. The results are recorded in the table. During effective therapy with androgen alone, the BMR rose to +10 per cent.

Effects of therapy: A 25-year-old man with preadolescent eunuchoidism received unesterified testosterone sublingually as his initial androgenic therapy. There was no response with 24 mg. daily but 48 mg. gave full effects. Maintenance was possible with 20 mg. of unesterified testosterone or 10 mg. of methyltestosterone sublingually per day, but testosterone propionate in carbowax was inert in a dose of 20 mg. per day.

DISCUSSION

By reference to Table 1, it will be observed that all three testosterone compounds produced androgenic effects when administered buccally or sublingually in tablet form. The various compounds, however, showed a definite quantitative variation in efficacy.

Excessive effects appeared only with methyltestosterone in doses of 10 to 40 mg. per day (Cases 3, 5, 8, 9, and 10). The same or larger doses of testosterone or testosterone propionate, in any of the bases used, did not produce an excessive effect.[6] In Case 11 a daily dose of 60 mg. of testosterone propionate in the wax base was completely ineffective, whereas 20 mg. per day of methyltestosterone in the lactose base produced a full androgenic response.

Comparison of methyltestosterone with unesterified testosterone indicated greater reliability and effectiveness of the methyl ester. In Case 8, it was seen that doses of 2.5 to 5 mg. daily of methyltestosterone linguets were as effective as 10 to 20 mg. daily of unesterified testosterone in the lactose or gelatin-sucrose base. In Case 9 the amounts were more comparable, with 10 to 15 mg. of methyltestosterone in the lactose base producing a full effect similar to that resulting from 10 to 20 mg. of unesterified testosterone in the same base. Twenty milligrams of methyltestosterone produced an excessive effect in the same patient. In Case 10, 10 to 20 mg. of unesterified testosterone in the lactose or gelatin-sucrose base caused a slight to moderate androgenic effect, whereas 10 mg. of methyltestosterone

[6] We have subsequently encountered occasional excessive effects from unesterified testosterone in the gelatin-sucrose base in doses of 24 mg. per day.

in lactose caused full to excessive effects. In Case 11, initial therapy with unesterified testosterone in doses of 30 mg. per day, in both the lactose and wax bases, was without effect, but 20 mg. of methyltestosterone in the lactose base produced a full androgenic effect. It was subsequently found that 18 mg. of unesterified testosterone in the gelatin-sucrose base produced a moderate androgenic effect, suggesting some increase of effectiveness when this base was used. In Case 12, 10 mg. of methyltestosterone produced a full androgenic effect comparable to that produced by 20 to 48 mg. of unesterified testosterone in the lactose or gelatin-sucrose base.

In cases in which it was possible to compare unesterified testosterone with testosterone propionate, it was found that testosterone propionate was less effective. In no case was more than a moderate effect obtained with testosterone propionate, and in Case 11 neither unesterified testosterone nor testosterone propionate in the lactose or the wax base had any effect in initiating an androgenic response, even in doses of 30 to 60 mg. daily. In Case 12, 20 mg. per day of testosterone propionate in the carbowax base produced no effect, whereas a full androgenic effect was obtained from 20 mg. per day of unesterified testosterone in lactose.

Thus, in this series of hypogonadal males, methyltestosterone was the most effective; unesterified testosterone was second in potency; and testosterone propionate had the least androgenic effect. The contrast is therefore most marked when the effects of methyltestosterone are compared with those of testosterone propionate. This is striking in Case 1, in which instance 20 mg. per day of testosterone propionate in lactose produced only a doubtful androgenic effect, whereas half that amount of methyltestosterone linguets (10 mg. per day) produced a full androgenic response. Case 8 showed a similar result, as 10 to 20 mg. per day of testosterone propionate in lactose produced a moderate androgenic effect which was matched by only 2.5 mg. per day of methyltestosterone linguets. In Case 9, 10 mg. per day of testosterone propionate in lactose produced only a moderate androgenic effect which was equaled by but 5 mg. per day of methyltestosterone linguets. The patient in Case 10 showed only a slight androgenic effect from 10 mg. per day of testosterone propionate in the lactose or the wax base, but the same dose of methyltestosterone linguets resulted in a full to excessive androgenic effect. In Case 11, testosterone propionate in both wax and lactose bases, with doses up to 60 mg. per day, failed to initiate an androgenic response, whereas 10 to 20 mg. per day of methyltestosterone linguets (lactose base) resulted in a full androgenic effect.

The superior effectiveness of methyltestosterone sublingually is probably related to the fact that the methyl group in the 17-position protects the steroid against inactivation by the liver. Absorption across the oral

mucosa into the general circulation protects only against initial inactivation by the liver. The 17-methyl ester is the compound that best survives subsequent hepatic inactivation and therefore has a longer time to exert its androgenic effects.

There is further evidence that the superior effectiveness of the sublingual route is related to circumventing initial inactivation of the compound by the liver, rather than to any increased absorption by this route. Swallowed, sublingual, and injected testosterone each increase urinary 17-ketosteroid excretion to the same degree, indicating equal absorption (14). Swallowed testosterone, however, does not produce androgenic or metabolic effects. If liver damage were present, the relative efficacy of these compounds might be expected to be different.

Regarding the effectiveness of the various bases, it has been known for a number of years that the lactose base is a satisfactory vehicle. Comparison with the more recently developed carbowax base (polyethylene glycol), as in Cases 8, 10, 11, and 12, indicates an equivalent effect. The gelatin-sucrose base, however, did seem to be somewhat superior to the lactose and wax bases in Cases 10 and 11. On the other hand, in Cases 8 and 12 the effects were similar. These comparisons were made only with unesterified testosterone.

It has been our experience, in this study and elsewhere, that the effects produced by the methyltestosterone linguets are the same, whether the tablet is dissolved under the tongue or in the buccal gutter. Difficulties with salivation or unpleasant taste have not been encountered with any of the buccal or sublingual tablets. The hard compressed tablet with slow dissolution is apparently essential to insure absorption across the oral mucosa. This is well illustrated in Case 4, when the patient accidentally received oral tablets of methyltestosterone designed to be swallowed and rapidly dissolved. When he took 10 mg. per day of this preparation sublingually, his androgenic status regressed from a full effect to a slight effect. Upon resuming the same dose sublingually with the hard compressed methyltestosterone linguet in lactose, he again experienced a full androgenic effect.

Whenever treatment was discontinued, or when placebos were substituted, there was a definite and prompt loss of androgenic effect. When chorionic gonadotropin was administered subcutaneously in a dose of 1,000 I.U. three times weekly for two months (Case 7), a moderate androgenic effect was produced. A full androgenic response was shown by the same patient with methyltestosterone linguets taken sublingually in a dose of 10 mg. per day.

It will be noted in Table 1 that this group of hypogonadal males showed a full androgenic effect during therapy with 10 mg. of methyltestosterone

linguets per day. This corresponds to a weekly dose of 70 mg., an amount comparable to the 75 mg. per week recommended by Thompson for maintenance with parenteral testosterone propionate (15).

SUMMARY

The androgenic effects of buccal tablets of testosterone, testosterone propionate, and methyltestosterone were assayed in 12 hypogonadal men. In 7 of the patients, treatment with tablets under the tongue or in the buccal gutter was the initial androgenic therapy. The other 5 had previously received parenteral androgens, but treatment had lapsed before the start of this study in 3 cases. Various preparations were compared serially in the same patient, and the degree of androgenic effect tabulated.

When administered as buccal or sublingual tablets, methyltestosterone was approximately twice as potent per milligram as unesterified testosterone, which in turn was approximately twice as potent as testosterone propionate. Failures to initiate or to maintain an androgenic effect were not encountered with methyltestosterone, but occasionally were found with the other two preparations. The type of base or the place in the buccal cavity in which the tablets were dissolved did not seem to alter the clinical effectiveness to any important degree. The gelatin-sucrose base seemed occasionally slightly superior to the lactose base or the wax base as a vehicle for unesterified testosterone. Hard compressed tablets designed for slow dissolution appear to be essential for full effectiveness by this route. In this series of hypogonadal men, 10 milligrams of methyltestosterone per day administered as a buccal or sublingual tablet was usually a full maintenance dose.

REFERENCES

1. JOËL, C. A.: Clinical results obtained with sublingual administration of methyl testosterone, *J. Clin. Endocrinol.* **2**: 116–119 (Feb.) 1942.
2. SPENCE, A. W.: Sublingual administration of methyl testosterone, *Brit. M. J.* **1**: 668 (May 30) 1942.
3. LISSER, H.; ESCAMILLA, R. F., and CURTIS, L. E.: Testosterone therapy of male eunuchoids. III. Sublingual administration of testosterone compounds, *J. Clin. Endocrinol.* **2**: 351–360 (June) 1942.
4. LISSER, H., and CURTIS, L. E.: Testosterone therapy of male eunuchoids. IV. Results from methyl testosterone linguets, *J. Clin. Endocrinol.* **3**: 389–399 (July) 1943.
5. LISSER, H., and CURTIS, L. E.: Treatment of post-traumatic Simmonds' disease with methyl testosterone linguets, *J. Clin. Endocrinol.* **5**: 363–366 (Nov.) 1945.
6. DUNN, C. W., and HOFFMAN, R. H.: Male hormone therapy, *Clinics* **5**: 878–897 (Dec.) 1946.
7. FINKLER, R. S.: Male hypogonadism treated by sublingual methyltestosterone, *J. Clin. Endocrinol.* **7**: 293–302 (April) 1947.
8. DEAMER, W. C.: Stimulation of growth in boys by sublingual (methyl) testosterone therapy, *Am. J. Dis. Child.* **75**: 850–859 (June) 1948.

9. Escamilla, R. F.: Treatment of preadolescent eunuchoidism with (methyl) testosterone linguets, *Am. Pract.* **3**: 425–427 (March) 1949.
10. Walton, R. P.: Sublingual administration of drugs, *J.A.M.A.* **124**: 138–143 (Jan. 15) 1944.
11. Anderson, E.; Haymaker, W., and Henderson, E.: Successful sublingual therapy in Addison's disease, *J.A.M.A.* **115**: 2167–2168 (Dec. 21) 1940.
12. Biskind, G. R.; Escamilla, R. F., and Lisser, H.: Implantation of testosterone compounds in cases of male eunuchoidism, *J. Clin. Endocrinol.* **1**: 38–49 (Jan.) 1941.
13. Escamilla, R. F., and Lisser, H.: Testosterone therapy of eunuchoids. II. Clinical comparison of parenteral implantation, and oral administration of testosterone compounds in male eunuchoidism, *J. Clin. Endocrinol.* **1**: 633–642 (Aug.) 1941.
14. Gordan, G. S., and Halliday, N.: Unpublished observations.
15. Thompson, W. O.: Uses and abuses of the male sex hormone, *J.A.M.A.* **132**: 185–188 (Sept. 28) 1946.

HORMONAL FACTORS PRODUCING THE GAMETOKINETIC RESPONSE IN THE MALE FROG (*RANA PIPIENS*)*

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SINCE the discovery by Galli Mainini (1, 2, 3, 4) that human pregnancy urine and chorionic gonadotropin will cause the release of spermatozoa by the male toad, *Bufo arenarum* Hensel, numerous species of male toads and frogs have been evaluated for their efficacy as pregnancy test animals (5–12).

The male leopard frog, *Rana pipiens*, has been widely used in this country as a pregnancy test animal following the procedure outlined by Miller and Wiltberger (6, 7) and Robbins and Parker (8). Robbins and Parker (13) have also shown that the South African male frog, *Xenopus laevis*, and the male frog, *Rana pipiens*, respond to the subcutaneous administration of epinephrine by the emission of spermatozoa, the *Xenopus laevis* frog being reactive throughout the year, the *Rana pipiens* frog passing through a refractory period during the summer months of June, July and August. Synthetic as well as naturally derived epinephrine proved to be equally effective in producing the release of spermatozoa; hypophysectomy failed to alter the reactivity of the animal to epinephrine.

In our laboratory we have compared the reliability of the *Rana pipiens* test to that of the rat ovarian hyperemia test for the diagnosis of early pregnancy (14). We have also determined the approximate minimal dose of the various hormone preparations (pituitary gonadotropin, prolactin, chorionic gonadotropin, equine gonadotropin, FSH and ICSH) necessary to produce emission of spermatozoa in the male frog, *Rana pipiens*, and ovarian hyperemia in the immature Sprague-Dawley rat.

EXPERIMENTAL METHODS

Test Animals: Medium sized adult male frogs (*Rana pipiens*) weighing approximately 25 to 45 grams were used in this study. Urine, serum, hormone preparations and drugs tested were injected into the dorsal lymph

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sac of the frog. If the injected frog gave a positive reponse, several days were allowed to elapse before using this frog again. Isotonic saline was used as the diluent for the injected gonadotropin preparations, ACTH (Armour), and powdered prolactin FWP28–29 r (Armour). In every case, urine was aspirated from the frog's cloaca and examined for the presence of spermatozoa prior to the injection of the test material. On the average, the frog's urine was checked for the presence of spermatozoa thirty minutes to three hours after the injection of the test substance. Each frog was kept in an isolation jar during the time its response to the various test materials was being measured. It was found that the more potent the gonadotropic substance injected, the more rapid the gametokinesis (at times within thirty to thirty-five minutes).

The rats used in this study were immature female rats of the Sprague-Dawley strain, weighing from 30 to 70 grams and varying in age from 21 to 30 days. Isotonic saline served as diluent for the gonadotropin preparations and the powdered prolactin FWP28–29 r (Armour). All of the test substances were injected intraperitoneally. Animals were asphyxiated with ether two to six hours after the injection of the test material and their ovaries examined for the presense of hyperemia according to the method of Kupperman and Greenblatt (15–19).

The hormone preparations employed in this study were:

1. Pituitary gonadotropin (Squibb—of equine pituitary origin)
2. Pituitary gonadotropin (Searle—Gonadophysin)
3. Combined anterior pituitary and chorionic gonadotropin (Parke, Davis & Company—Synapoidin)
4. Chorionic gonadotropin (Ayerst, McKenna & Harrison—A.P.L.)
5. Pregnant mare's serum gonadotropin (Ayerst, McKenna & Harrison—Antex)
6. Special prolactin (Armour—in solution containing 0.5 per cent phenol; slight traces of gonadotropin possibly present)
7. Prolactin FWP28-29 r (Armour—in powdered form; slight traces of gonadotropin possibly present)
8. ICSH FW234 (Armour—mainly of LH type but containing traces of prolactin, thyrotropin, growth hormone, ACTH, and a small amount of posterior lobe hormones)
9. Follicle-stimulating hormone (Schering—FSH, containing a small amount of LH and thyrotropin, and traces of prolactin)
10. Follicle-stimulating hormone, FSH 147HM3 (supplied by Dr. W. H. McShan, Associate Professor of Zoochemistry, University of Wisconsin, 8.8 milligrams of the material being equivalent to 1 gram of dry pituitary powder)

It was found necessary to inject fresh hormone preparations, as old preparations were of considerably lower potency. Large numbers of rats and frogs were injected in order to arrive at the approximate minimal dose necessary to produce a positive response *i.e.*, gametokinesis (release of spermatozoa by the male frog) and ovarian hyperemia in the immature female rat.

COMPARISON OF RESPONSE OF MALE FROG AND OF FEMALE RAT TO HORMONE PREPARATIONS

As seen in Table 1, the immature female rat was more sensitive to small amounts of prolactin, pituitary gonadotropin, ICSH, combined anterior pituitary and chorionic gonadotropin, chorionic gonadotropin and FSH (Schering) than the male frog. The male frog gave a positive response with 25 I.U. of pregnant mares' serum, whereas 35 I.U. were necessary to evoke rat ovarian hyperemia. Ten milligrams of FSH 147HM3 (McShan) failed to produce rat ovarian hyperemia, whereas a weak positive response was obtained in the male frog at this dose level. It would appear from this evidence that the rat ovarian hyperemia response is due to the LH factor and is augmented by the presence of FSH, confirming the findings of Rakoff and Fried (20) and Kupperman, McShan and Meyer (21). The gametokinetic response seems to depend primarily on FSH rather than on LH.

Control experiments: Urine and/or serum from patients exhibiting varying clinical syndromes was injected into the male frog to determine whether gametokinesis could be produced. In only 1 case (in a patient with myxedema, fifteen minutes following completion of the epinephrine infusion in the test for pituitary insufficiency as devised by Recant, Forsham and Thorn (22)) did the urine and serum cause release of spermatozoa in the male frog.

Also the following substances were injected into the dorsal lymph sac of the male frog, *Rana pipiens*, with a resulting negative response: 0.05 mg. and 0.1 mg. of Prostigmin; 0.1 mg. and 0.2 mg. of histamine phosphate; 1.0 mg. of dexedrine; 10.0 mg. of neosynephrine, and 5.0 mg. of ACTH (Armour).

SUMMARY AND CONCLUSIONS

Various commercial hormone preparations (pituitary gonadotropin, chorionic gonadotropin, combined anterior pituitary and chorionic gonadotropin, pregnant mare's serum gonadotropin, prolactin, interstitial cell-stimulating hormone, follicle-stimulating hormone) and FSH 147HM3 (McShan) were injected into the male frog, *Rana pipiens*, and into the immature female Sprague-Dawley rat in order to determine the approximate minimal dose necessary to cause gametokinesis and ovarian hyperemia respectively. A much smaller quantity of the commercial hormone extracts was needed to evoke rat ovarian hyperemia than to induce gametokinesis, except in the case of pregnant mare's serum gonadotropin. Purified FSH 147HM3 caused gametokinesis but 10 mg. of the material failed to produce rat ovarian hyperemia. The rat ovarian hyperemia response appears to be due to the LH factor and to be augmented by the FSH factor. The gametokinetic response in the male frog, *Rana pipiens*, seems to depend primarily on the FSH factor rather than on the LH factor.

TABLE 1. APPROXIMATE MINIMAL DOSE OF HORMONES PRODUCING GAMETOKINESIS AND OVARIAN HYPEREMIA

Hormone	Minimal dose producing gametokinesis in male frogs (*Rana pipiens*)	Minimal dose producing ovarian hyperemia in female rat (Sprague-Dawley)
Pituitary gonadotropin of equine origin (Squibb)	2.5 R.U.	0.02 R.U.
Pituitary gonadotropin (Gonadophysin, Searle)	0.5 R.U.	<0.005 R.U.
Combined anterior pituitary and chorionic gonadotropin (Synapoidin, Parke Davis)	0.5 S.R.U.	0.02 S.R.U.
Chorionic gonadotropin (A.P.L., Ayerst)	15 I.U.	2.0 I.U.
Pregnant mare's serum gonadotropin (Antex, Ayerst)	25 I.U.	35 I.U.
Special prolactin (Armour)	100 I.U.	4.0 I.U.
Prolactin(FWP 28–29r, Armour)	<94.5 ±23.1 I.U.	6.8 I.U. ±1.71 I.U.
Interstitial cell-stimulating hormone (FW234, Armour)	15.9 Collip units	3.2 Collip units
Follicle-stimulating hormone (FSH, Schering)	0.5 S.U.	0.2 S.U.
Follicle-stimulating hormone (FSH 147HM3, McShan)	<10 mg.	No hyperemia with 10 mg.

Urine and/or serum from patients with various clinical syndromes failed to produce the gametokinetic response in the male frog except in the case of 1 patient with myxedema who had received an infusion of 1.5 mg. of epinephrine. Prostigmin, histamine phosphate, dexedrine, neosynephrine, and ACTH (Armour) failed to produce gametokinesis in the dose employed.

This comparative study was conducted during the winter and spring months. Preliminary observations indicate that the male frog, *Rana pipiens*, is less sensitive to chorionic gonadotropin during the summer months.

REFERENCES

1. Galli Mainini, C.: Reacción diagnóstica de embarazo en la que se usa el sapo macho como animal reactivo, *Semana méd.* **54:** 337 (March 20) 1947.
2. Galli Mainini, C.: Reacción diagnóstica de embarazo y acción de las gonadotrofinas en el sapo macho, *Semana méd.* **54:** 447 (Sept. 18) 1947.
3. Galli Mainini, C.: Pregnancy test using the male toad, *J. Clin. Endocrinol.* **7:** 653 (Sept.) 1947.
4. Galli Mainini, C.: Pregnancy test using the male Batrachia, *J.A.M.A.* **138:** 121 (Sept. 11) 1948.
5. Galli Mainini, C.: Pregnancy test with male Batrachia, *Endocrinology* **43:** 349 (Nov.) 1948.
6. Wiltberger, P. B., and Miller, D. F.: The male frog, *Rana pipiens*, as a new test animal for early pregnancy, *Science* **107:** 198 (Feb. 20) 1948.
7. Miller, D. F., and Wiltberger, P. B.: Some peculiarities of the male frog test for early pregnancy, *Ohio J. Sci.* **48:** 89, 1948.
8. Robbins, S. L., and Parker, F., Jr: The use of the male North American frog (Rana pipiens) in the diagnosis of pregnancy, *Endocrinology* **42:** 237 (April) 1948.
9. Brody, H.: The use of the male leopard frog (Rana pipiens) as a pregnancy test animal, *Am. J. Obst. & Gynec.* **57:** 581 (March) 1949.
10. McCallin, P. F., and Whitehead, R. W.: A study of native species of male toads as test animals in the diagnosis of early human pregnancy, *Science* **109:** 518 (May 20) 1949.
11. Bhaduri, J. L., and Bardhan, N. R.: Male frogs and toads as test animals for early pregnancy and certain related conditions, *Science* **109:** 517 (May 20) 1949.
12. Nobili, L.: Sulla reazione biologica di Galli Mainini per la diagnosi de gravidanze. Prime osservazioni, *Riv. ital. di ginec.* **31:** 352, 1948. (Reviewed in International Abstracts of Surgery **88:** 518, 1949.)
13. Robbins, S. L., and Parker, F., Jr.: The reaction of male frogs to epinephrine, *Endocrinology* **44:** 384 (April) 1949.
14. Greenblatt, R. B.; Clark, S. L., and West, R. M.: The male frog (Rana pipiens) compared to the female rat as a pregnancy test animal, (in press).
15. Kupperman, H. S.; Greenblatt, R. B., and Noback, C. R.: A two and six-hour pregnancy test, *J. Clin. Endocrinol.* **3:** 548 (Oct.) 1943.
16. Kupperman, H. S., and Greenblatt, R. B.: A rapid test for pregnancy and its evaluation as compared with other known tests, *Bull. Univ. Hosp.* (Augusta, Ga.) **5:** 6, 1944.
17. Kupperman, H. S., and Greenblatt, R. B.: The two hour pregnancy test, *South. M. J.* **39:** 158, 1946.
18. Kupperman, H. S., and Greenblatt, R. B.: Further observations on the two hour pregnancy test, *J. M. A. Georgia* **33:** 58, 1947.
19. Kupperman, H. S., and Greenblatt, R. B.: The two-hour test for the early diagnosis of pregnancy, *Postgrad. Med.* **4:** 519, 1948.
20. Rakoff, A. E., and Fried, P. H.: Enhancement of effect of chorionic gonadotrophin on ovarian hyperemia in the rat by pituitary extract, *Proc. Soc. Exper. Biol. & Med.* **66:** 491 (Nov.) 1947.
21. Kupperman, H. S.; McShan, W. H., and Meyer, R. K.: Gonadotrophic hormones and ovarian hyperemia in the rat, *Endocrinology* **43:** 275 (Nov.) 1948.
22. Recant, L.; Forsham, P. H., and Thorn, G. W.: Observations on the pituitary-adrenal response following epinephrine infusion in man, *J. Clin. Endocrinol.* **8:** 589, 1948.

HIRSUTISM AND VIRILISM IN A 5-YEAR-OLD GIRL

REMISSION FOLLOWING REMOVAL OF ADRENAL CARCINOMA: RECURRENCE AFTER THREE AND A HALF YEARS

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IN 1939 Reilly, Lisser and Hinman (1), in a report of one of their own cases of pseudo pubertas praecox, included results of a search of the literature for all known cases of pseudo pubertas praecox in girls, in which the diagnosis was not open to doubt. It was found that without a single exception the condition in these cases was caused by tumors or hyperplasia of the adrenal cortex. So far as we know, no cases in which the underlying cause was different from the foregoing were published during or after the recent war (2). Twenty of the 40 patients in the cases collected by Reilly, Lisser and Hinman were operated upon; only 10 were cured.

CASE REPORT

The patient was first seen in February, 1944, at the age of 5 years and 4 months. She had been born at full term on September 5, 1937. It was said that at birth she had exhibited a rather extensive growth of hair. Apart from this, however, she grew up in good health and was completely normal until shortly before we saw her. In photographs taken before her illness developed she looked normal (Fig. 1). Except for measles and acute cystitis, the patient had not been ill before. At the age of 5 years she had become obese. The breasts had begun to develop and a heavy growth of hair had appeared over the entire body. Her manner of talking had become very "old for her age," and her voice had deepened. Menstruation did not occur. During the three months prior to her admission she had experienced increasing abdominal pains, occasionally accompanied by a feeling of sickness and vomiting.

Clinical features.—The patient impressed us as being a kindly, dark-haired, miniature woman, far in advance of her age both physically and mentally. She behaved like an adult person rather than a child, and joined in older people's conversation in an assured natural manner. Her vocabulary was not that of a child at all: when, for example, citing an incident in which her mother had protected her with bare arms against broken glass from a smashed automobile, she declared: "May I never lose the recollection of that brave action!" She had not the slightest hesitation in displaying her affection for one of the physicians; whenever possible she would rush at him and throw her short, muscular arms about his neck in an intimate embrace. There were no signs of any direct sexual urge or of homosexuality. She was very fat, especially on the back, neck, breasts and abdomen; arms and legs also were fat (Fig. 2, *a* and *b*).

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The glandular tissue of the breasts had increased slightly, but the breasts consisted chiefly of fat. The areolae were juvenile. Hirsutism was most pronounced on the back, especially in the loins and neck, where the hair was short, thick and black. The pubic hair was of masculine distribution; there was hair in the left axilla. The patient had a small mustache, distinct side whiskers and a pimpled face. The skin was coarser than is usual in persons of her age.

The labia minora were large; the clitoris was very large. The uterus could not be

FIG. 1. The patient at the age of 3 years, apparently a perfectly normal child.

palpated rectally. The musculature was strongly developed. The teeth were changing prematurely, both upper and lower medial incisors already had been replaced. The thyroid gland could not be felt; the patient had a short fat neck. The thorax and abdomen exhibited no abnormalities except for overdeveloped panniculus adiposus. Reflexes were normal. Systolic blood pressure was 105 and diastolic, 80, expressed in millimeters of mercury. Her weight on February 4, 1944, was approximately 62 pounds (28 Kg.).

Examination of the patient was continued with the following objectives: 1) collection of all possible data in regard to abnormal metabolism and disturbed hormonal balance; 2) discovery of the cause of these disturbances, with a view to surgical removal of them; 3) estimation of the chances for successful operation, determination of the

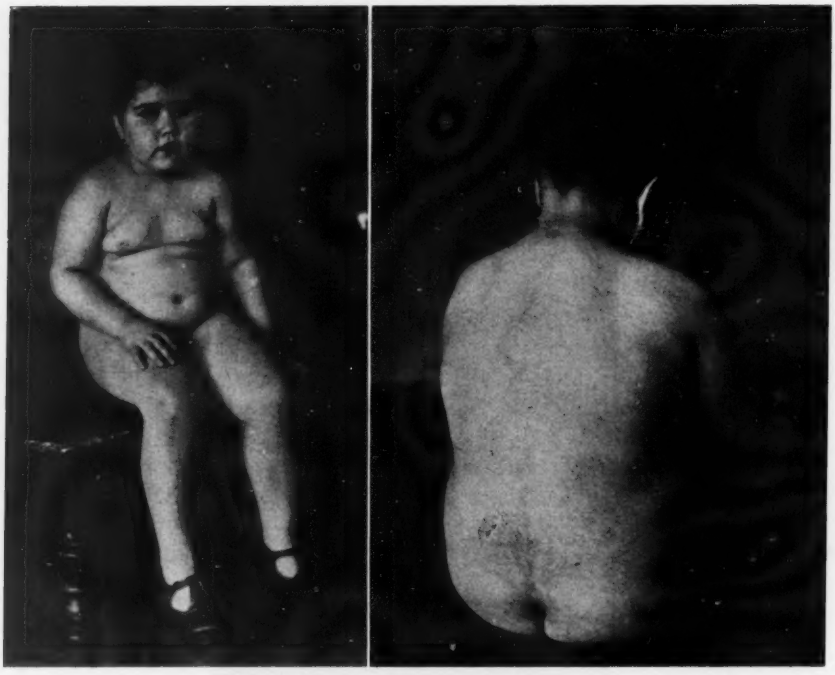

FIG. 2. *a* and *b*. The patient in February, 1944, at the age of 5 years and 4 months. Note the dark hair, obesity and growth of hair along the spinal ridge.

TABLE 1. STUDIES OF THE BLOOD BEFORE AND AFTER OPERATION FOR HIRSUTISM AND VIRILISM

	Before operation		After operation	
	Date	Mg. per 100 cc.	Date	Mg. per 100 cc.
Cholesterol	Feb. 4	96 to 97	May 25	133 to 143
Cholesterol	17	182 to 192		
Chlorides (as sodium chloride)	17	644 to 645	25	622 to 624
Calcium	17	10.8 to 11.2	25	11.6 to 11.7
Nonprotein nitrogen	17	27 to 33	25	18
Potassium	23	19.1	25	17.1 to 17.6
Sodium	23	339 to 348	25	346 to 348
Phosphate	April 3	3.7	25	6.9

presence or absence of metastasis, and of any contraindications to surgical treatment.

Blood, metabolism, and hormone excretion studies.—Results of studies of the blood are seen in Table 1. The mineral and protein spectra were normal. The blood sugar curve, after administration of 25 grams of glucose, showed a slight inclination toward hypoglycemia. Two and a half hours after the injection of glucose and after the administration of insulin and epinephrine, the blood sugar curves showed nothing abnormal.

The metabolic rate on February 4, 1944, was +14 per cent.

Determinations of the content of hormones in the urine were made before the operation. An estrogen content of 60 international units per liter was found, which is rather high for a girl of 5½ years. The androgen content was 20 international units per liter. Contrary to expectations based on the clinical picture, this did not increase.

A roentgenogram of the hands showed nothing abnormal in the epiphyseal lines.

Etiologic investigation.—Our first line of inquiry was in the direction of a possible adrenal tumor. Intravenous pyelograms strongly suggested a small tumor shadow, containing a calcareous spot, above the right kidney. This shadow was projected in a transverse view at the level of the first thoracic vertebra.

Perirenal insufflation was carried out on March 6, 1944, according to the method of Cope and Schatzki (3). Both kidneys were clearly visible, although air was insufflated on the right side only. The right adrenal gland appeared enlarged; this was not distinctly visible in the transverse views.

In planigrams made after insufflation, the right adrenal body appeared to be enlarged, with a calcareous shadow in the center. The enlarged right adrenal body lay ventrad and mediad above the kidney. The left adrenal body also was visible; it was uncertain whether or not it was similarly enlarged.

Hence, all our findings indicated the adrenal bodies to be the site of the basic condition; the likelihood of a tumor in the right adrenal body was especially strong. Yet, neither upon palpation nor rectal examination could any semblance of this tumor or of other tumors be felt which might have been situated in the abdomen. A roentgenogram of the skull showed a normal sella turcica and no abnormal shadows in the region of the epiphysis. The neurologist found no abnormalities; the visual fields appeared normal.

Prognosis.—Adrenal tumors in children nearly always are carcinomas, and frequently are bilateral. We found no trace of any form of metastasis. The left adrenal body was not entirely free from suspicion, in view of the planigram. It was decided to intervene surgically.

Operation.—On April 4, 1944, the right adrenal body, which was the size of a walnut, was easily removed by Dr. Kehrer. It proved to be a carcinoma of the adrenal cortex. By means of ponceau-fuchsin staining, the "specific" Vines' granules were found (4).

Postoperative course.—The pulse rate and temperature are depicted in Figure 3. The course was stormy, with vomiting, low blood pressure, high pulse rate and high temperature. Intravenous drip infusion was instituted and 10 cc. of Cortin was injected shortly after the operation. This probably did no good, for an hour later the potassium content of the serum was only 9.9 mg. per 100 cc. and the content of sodium was between 348 and 350 mg. per 100 cc. These values were not determined until the next day, by which time 60 cc. of Cortin already had been given intravenously. On April 5 the value for potassium was 4.3 to 5.1 mg. per 100 cc. of serum, and that for sodium was 328 to 335 mg. per 100 cc. of serum. No more Cortin was administered after this; potassium chloride was given in a dose of 2 Gm. per hour. The potassium content of the serum improved to 12 mg. per 100 cc. on April 6 and to 19.3 mg. per 100 cc. on April 7. The appearance of the patient twenty-five days after the operation is seen in Figure 4.

One month after the operation there was a renewed increase in the pulse rate, accompanied by vomiting (Fig. 3) and after this, also, an increase in temperature to 40.3° C. (104.5° F).

On April 30 the urine was found to contain acetone and diacetic acid, but no glucose. The alkaline reserve on April 29 was 22 volumes per cent. Although Cortin was not injected during this time, the potassium content of the serum decreased again, this time to 11.7 mg. per 100 cc. Hence, the conditions we saw at this time were acetonemia, vomiting and hypopotassemia, accompanied by a very rapid pulse, hypotension and high temperature, without any other traceable cause. This attack was treated successfully by drip infusion, but not without considerable trouble and anxiety. During the following months

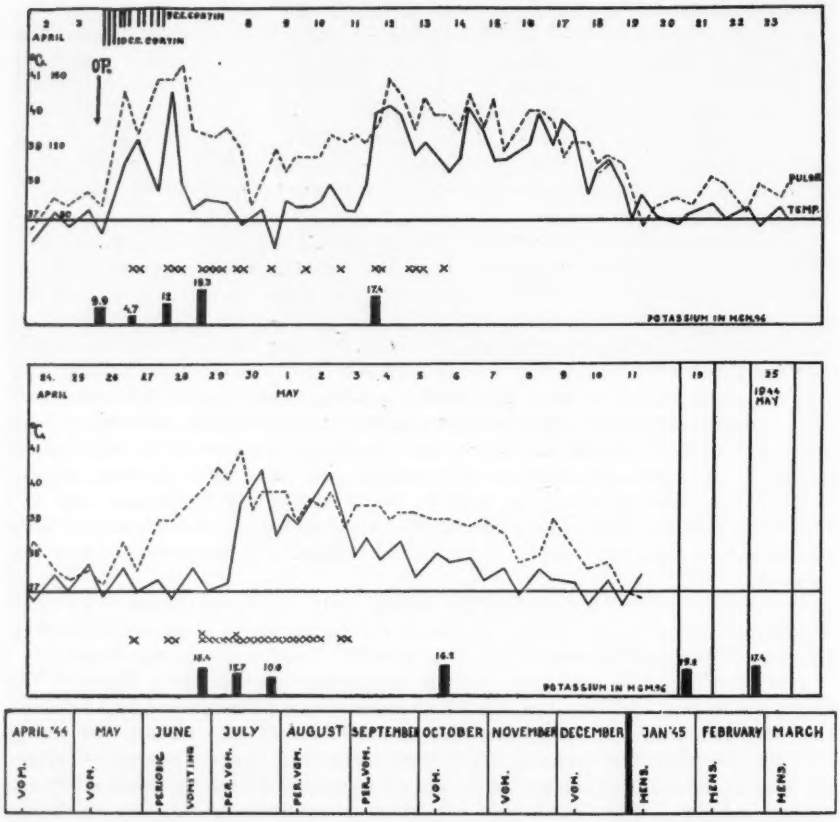

FIG. 3. Showing pulse, temperature and serum potassium values through stormy postoperative course after right adrenalectomy (OP.) performed on April 4. X's mark episodes of vomiting. The dated compartments at the bottom of the chart show the occurrence of vomiting from April, 1944, through March, 1945. Note how the periodic episodes of vomiting gradually merged into regular menstruation in January, 1945, when the patient was 6½ years old.

these attacks of violent vomiting, at times accompanied by severe abdominal pain, recurred periodically. They are indicated in the dated compartments in the lower part of Figure 3. The attacks finally began to recur at regular intervals of a month, and gradually came to coincide equally regularly with menstrual periods. The patient was then 6½ years old.

FIG. 4. The patient twenty-five days after right adrenalectomy, showing operative incision.

Disappearance of hirsutism after operation.—On June 20, 1944, two and a half months after the operation, the patient came to us for re-examination. She appeared to be a different person. The superfluous hair was falling out; when she washed, it adhered to her washcloth. She had grown thinner; her voice was higher pitched and more childlike. Her face was becoming that of a normal child again. Her deportment was childlike; she no longer joined in older people's conversation. She sucked her thumb, and wanted her mother to "tuck her in" at night, a thing which formerly had caused her to ridicule her little brother. Her skin was becoming soft and supple instead of coarse and harsh; the pimples on her face had disappeared; her eyebrows had grown thinner; the mustache and

the side whiskers had disappeared. The pubic hair was straggling and scanty. The mammary disks were more palpable than before, since at this time her weight was less. The clitoris was still larger than would be expected in a person of her age, but was nonetheless distinctly smaller than it had been. It could not, as was the case previously, be seen protruding between the labia. The weight of the patient at this examination was approximately 56 pounds (25.4 Kg.).

FIG. 5. The patient on April 22, 1945, one year after the operation, at the age of 6½ years. The skin and the hair were soft and hirsutism had disappeared, but the mammary disks were palpable.

The patient's urine was assayed by Dr. Dingemanse at Amsterdam after the operation; no excess of any 17-ketosteroid was found (5). The appearance one year after operation is seen in Figure 5.

Later years.—As before mentioned, ten months after the operation menstruation started regularly. In the patient's seventh and eighth years, then, she was confronted with physiologic problems which other children do not encounter until the age of 13 to 17 years. She was able to take her mother's place at home in many ways. She manifested some interest in sexual problems and in boys, and did not play with girls of her own age. Rather, she preferred to talk to adult persons. She still tired easily, often complained of

abdominal pain and, when it was cold, had clammy hands and feet. In place of the coarse hair which had fallen out after the operation, there appeared a very soft, downy completely feminine growth of hair. Like her hair, her whole personality was entirely feminine.

In February, 1947, menstruation stopped. The patient's interest in boys disappeared. In August, 1947, there was again an abrupt change. The weight suddenly increased 2 pounds a week. Hirsutism returned. The patient again began to take part in the talk of grown-up people, and she discussed the weekly family budget earnestly.

FIG. 6. Roentgenogram of the patient made on December 19, 1947, which disclosed an enormous metastatic process in the field of the right lung and a tumor mass above the left side of the diaphragm and in the region of the left adrenal body.

On December 19, 1947, an enormous round metastatic process was seen roentgenologically in the right lung, a vast tumor mass with large shadows was visualized above the left side of the diaphragm and a shadow was noted in the region of the left adrenal body (Fig. 6). The value for serum potassium was normal: 18.5 to 20 mg. per 100 cc. The total amount of 17-ketosteroids excreted in twenty-four hours was 30 mg. (a normal adult woman excretes 5 to 17 mg. in this period.) Chromatographic analysis carried out by Dr. Dingemanse showed all the 17-ketosteroids to be increased. Androsterone and hydroandrosterone amounted to 54 international units. The basal metabolic rate was very low: −38 per cent. The blood sugar curve, after the administration of glucose and 4 units of insulin, was normal. Hirsutism again was marked (Fig. 7). The vulva was the size of that of a woman, but the clitoris was larger and hypospadic. On May 10, 1948, Dr. Dingemanse found that the excessive amount of 826 mg. of 17-ketosteroids was excreted in the urine in twenty-four hours. The value for estrogens per liter of urine was 600 international units.

The patient died at home in June, 1948, four years after operation and apparent surgical cure of a carcinoma of the right adrenal body.

FIG. 7. The patient in December, 1947, at the age of 9 years. A sudden change in her condition had occurred in August, 1947, in which hirsutism recurred and her weight began to increase at the rate of about 2 pounds a week.

SUMMARY

This report concerns a case of pseudo pubertas praecox with hirsutism, which developed in a girl 5 years old. A carcinoma of the right adrenal cortex was removed. After the operation there were severe attacks of vomiting, with hypopotassemia, acetonemia, very low blood pressure and shock. Less severe attacks recurred at regular monthly intervals. Menstruation, which started ten months after operation, gradually became equally regular when the patient reached the age of $6\frac{1}{2}$ years.

The patient's hirsutism, obesity and adult behavior disappeared after operation. She gradually became more nearly feminine, but both the feminine aspects and menstruation subsided again at the of $9\frac{1}{2}$ years. When the patient was 10 years old the full picture of pseudo pubertas praecox (but with enlarged mammae) recurred, with hirsutism. Extensive metastasis in the lungs, probably arising from a carcinoma of the left adrenal cortex, was found. The patient died at the age of $10\frac{1}{2}$ years.

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REFERENCES

1. Reilly, W. A.; Lisser, H., and Hinman, F.: Pseudo-sexual-precosity; the adrenal cortical syndrome in preadolescent girls: report of a successfully operated case, *Endocrinology* **24**: 91–114 (Jan.) 1939.
2. Selye, Hans: Textbook of Endocrinology. Montreal, *Acta Endocrinologica*, 1947, 914 pp.
3. Cope, O., and Schatzki, R.: Tumors of adrenal glands; modified air injection roentgen technic for demonstrating cortical and medullary tumors, *Arch. Int. Med.* **64**: 1222–1238 (Dec.) 1939.
4. Broster, L. R.; Vines, A., Patterson, G., and Butler, M.: The Adrenal Cortex Intersexuality. London, Chapman & Hall, Ltd., 1938, 257 pp.
5. Huis in't Veld, L. G., and Dingemanse, E.: Qualitatieve en quantitatieve bepaling van 17-ketosteroiden in urine langs coloriemtrischen weg, *Nederl. tijdschr. v. geneesk.* **90**: 666–671 (June 15) 1946.

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THE AMERICAN DIABETES ASSOCIATION

MIAMI BEACH, FLORIDA RONEY PLAZA HOTEL APRIL 3–8, 1950

The faculty will consist of prominent researchers and clinicians in the field of endocrinology and metabolic disorders, from the United States and Canada.

The course will be a practical one of interest and value to the specialist and those in general practice. The program will consist of lectures, clinics and demonstrations. Ample time will be given to questions and answers at the end of each session, and registrants are encouraged to contact members of the faculty for individual discussions.

The Roney Plaza, one of Miami Beach's most delightful hotels, offers special convention rates to members of this assembly. This is an unusual opportunity for you and your family to enjoy a pleasant vacation and for you to participate in a highly instructive program of the latest advances in endocrinology and metabolism.

A fee of $75 will be charged for the entire course and the attendance will be limited to 100. REGISTRATION WILL BE IN THE ORDER OF CHECKS RECEIVED AND WILL CLOSE ON MARCH 3, 1950. Should there be an insufficient number of applicants to fill the course, the registration fee will be refunded immediately in its full amount.

Application for approval of this course has been made to the Veterans Administration. Veterans should make formal application to their local agencies on the appropriate form (1905e or 1950) as furnished by the V.A.

Please forward application on your letterhead, together with check payable to The Association for the Study of Internal Secretions, to Henry H. Turner, M.D., Secretary-Treasurer, 1200 North Walker Street, Oklahoma City 3, Oklahoma, before March 3, 1950. Further information and program will be furnished upon request.

Hotel reservations should be made directly with the Roney Plaza Hotel, Miami Beach, Florida. and the hotel advised that you are attending this Postgraduate Assembly.

PROGRAM

Monday, April 3, 1950

8:30– 9:00 A.M. REGISTRATION

ENDOCRINE TERMINOLOGY AND METHODOLOGY

9:00– 9:30	The Biochemistry and Functional Interrelationships of the Endocrine Glands Dr. Kenyon
9:30–10:00	Regulation of Fat and Protein Metabolism Dr. Long
10:00–10:30	Nutrition and Hormones Dr. Long
10:30–10:45	INTERMISSION—REFRESHMENTS
10:45–11:15	Endocrine Laboratory Procedures Dr. Greenblatt
11:15–11:45	Evaluation of Laboratory Aids in Endocrine Diagnosis Dr. Browne
11:45–12:45 P.M.	Questions and Answers Dr. Kenyon, Moderator
12:45– 2:00	LUNCHEON

THYROID

Dr. Starr, Presiding

2:00– 2:30	Hypothyroidism in Children Dr. Turner
2:30– 3:00	Hypothyroidism in Adults Dr. Starr
3:00– 3:30	Hyperthyroidism—Physiopathology Dr. McCullagh
3:30– 3:45	INTERMISSION—REFRESHMENTS
3:45– 4:15	Hyperthyroidism—Diagnosis Dr. Haines
4:15– 4:45	Hyperthyroidism—Complications Dr. Starr
4:45– 5:15	Tumors of the Neck—Differential Diagnosis Dr. Lahey
	Questions and Answers deferred to Evening Session

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6:00	Get Acquainted Party—Cocktails and DINNER
7:30	Treatment of Hyperthyroidism Thyroidectomy.....Dr. Lahey Antithyroid Compounds.....Dr. Starr Radioactive Iodine.....Dr. McCullagh General Management.....Dr. Haines Questions and Answers Dr. Lahey, Moderator

Tuesday, April 4

GONADS—MALE

9:00– 9:30 A.M.	Physiopathology of the Testis Dr. Nelson
9:30–10:00	Sterility, Its Causes and Treatment Dr. Nelson
10:00–10:30	Hypogonadism Dr. Thompson
10:30–10:45	INTERMISSION—REFRESHMENTS
10:45–11:15	Gynecomastia Dr. Greenblatt
11:15–11:45	Use and Abuse of Sex Hormones Dr. Thompson
11:45–12:45 P.M.	Questions and Answers Dr. Thompson, Moderator
12:45– 2:00	LUNCHEON

GONADS—FEMALE

2:00– 2:30	Biochemistry and Physiopathology of the Ovary Dr. Nelson
2:30– 3:00	Hypogonadism and Infertility Dr. Hamblen
3:00– 3:30	Disorders of Menstruation Dr. Hamblen
3:30– 3:45	INTERMISSION—REFRESHMENTS
3:45– 4:15	Evaluation of Cervical and Vaginal Smears Dr. Greenblatt
4:15– 4:45	The Climacteric—Male and Female Dr. McCullagh
4:45– 5:15	Use of Sex Hormones in Malignancies Dr. Thompson
5:15– 5:45	Questions and Answers Dr. Hamblen, Moderator

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8:00	Demonstration of Laboratory Procedures in Endocrine Diagnosis Drs. Greenblatt and Nelson

Wednesday, April 5

PITUITARY

9:00– 9:30 A.M.	Physiopathology and Interrelationships Dr. Nelson
9:30–10:00	Hypopituitarism—Panhypopituitarism, Fröhlich's Syndrome; Simmonds' Disease and Anorexia Nervosa Dr. Kenyon
10:00–10:30	Hyperpituitarism—Gigantism and Acromegaly Dr. Kenyon
10:30–10:45	INTERMISSION—REFRESHMENTS

ADRENALS

10:45–11:15	Physiopathology Dr. Thorn
11:15–11:45	Adrenal Cortical Insufficiency Dr. Thorn
11:45–12:45 P.M.	Questions and Answers Dr. Rynearson, Moderator
12:45– 2:00	LUNCHEON
2:00– 2:30	Adrenal Cortical Hyperfunction (Cushing's Syndrome) Dr. Browne
2:30– 3:00	Adrenogenital Syndromes Dr. Turner
3:00– 3:30	Pheochromocytoma and Adrenergic Blocking Agents Dr. Thorn
3:30– 3:45	INTERMISSION—REFRESHMENTS
3:45– 4:15	The General Adaptation Syndrome Dr. Selye
4:15– 4:45	Diseases of Adaptation Dr. Selye
4:45– 5:15	The Adaptation Syndrome in General Medicine Dr. Browne
5:15– 5:45	Questions and Answers Dr. Thorn, Moderator

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8:00	ACTH, Cortisone and Other Compounds in the Treatment of Arthritis and Allied Conditions Dr. Thorn Questions and Answers Dr. Long, Moderator

Thursday, April 6

PARATHYROIDS

9:00– 9:30 A.M.	Hypo- and Hyperparathyroidism Dr. Rynearson
9:30–10:00	Calcium and Phosphorus Metabolism in Bone Disease Dr. Rynearson

GENERAL

10:00–10:30	Constitutional and Endocrine Factors in Retarded Growth Dr. Turner
10:30–10:45	INTERMISSION—REFRESHMENTS
10:45–11:15	Obesity and Thinness Dr. Rynearson
11:15–11:45	Endocrinology in General Medicine Dr. Browne
11:45–12:45 P.M.	Questions and Answers Dr. McCullagh, Moderator

12:45– 2:00 LUNCHEON

2:00 ENDOCRINE CLINICS
By members of Postgraduate Faculty. Clinics arranged by Dr. Carlos P. Lamar and Local Committee.

Friday, April 7

DIABETES MELLITUS AND DISEASES OF CARBOHYDRATE METABOLISM

9:00– 9:30 A.M. Modern Concepts of the Physiopathology of Diabetes Mellitus (New Developments in Experimental Diabetes)
Dr. Barach

9:30–10:00 Diagnostic Problems—Diabetes Detection
Dr. Root

10:00–10:30 Laboratory Procedures in Diabetology: Interpretation and Use of Blood Sugar Values—The Glucose Tolerance Test—The Urinalysis
Dr. Long

10:30–10:45 INTERMISSION—REFRESHMENTS

10:45–11:15 Clinical Aspects of Major Endocrine Disorders in Relation to Diabetes
Dr. McCullagh

11:15–11:45 How to Select and Prescribe an Adequate Diet and Instruct the Patient
Dr. Barach

11:45–12:15 P.M. Physiology of Insulin
Dr. Ricketts

12:15–12:45 Questions and Answers
Dr. Beardwood, Jr., Moderator

12:45– 2:00 LUNCHEON

2:00– 2:30 The Various Insulins and How to Use Them
Dr. Ricketts

2:00– 3:00 Insulin Problems: Resistance, Allergy, Local Dystrophies
Dr. Allan

3:00– 3:30 Acidosis and Coma
Dr. MacBryde

3:30– 3:45 INTERMISSION—REFRESHMENTS

3:45– 4:30 Diabetic Arteriosclerosis—Retinopathies—Wilson-Kimmestiel Syndrome
Dr. McCullagh

4:30– 5:15 Control of Diabetes
Dr. Root

5:15– 5:45 Questions and Answers
Dr. Root, Moderator

Saturday, April 8

9:00– 9:30 A.M. Diabetic Statistics—How to Use and Interpret Them
Dr. Root

9:30–10:00 Hyperinsulinism and Hypoglycemia
Dr. Beardwood, Jr.

10:00–10:30	Conservative and Surgical Management of Diabetic Gangrene Dr. Allan
10:30–10:45	INTERMISSION—REFRESHMENTS
10:45–11:15	Diabetes and Pregnancy Dr. Barach
11:15–11:45	The Diabetic Child Dr. MacBryde
11:45–12:15 P.M.	The Future of Diabetes—The American Diabetes Association and Its Affiliates Dr. Beardwood, Jr.
12:15–12:45	Questions and Answers Dr. Ricketts, Moderator
12:45– 2:00	LUNCHEON
2:00	DIABETES CLINICS By members of the Postgraduate Faculty. Clinics arranged by Dr. Carlos P. Lamar and Local Committee

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JEFFERSON MEDICAL COLLEGE AND HOSPITAL FELLOWSHIP IN GYNECOLOGIC ENDOCRINOLOGY

A fellowship in Gynecologic Endocrinology will be available in the Department of Obstetrics and Gynecology, Jefferson Medical College and Hospital, on July 1, 1950.

Applicants for the fellowship must have had at least a one-year residency or equivalent training in Obstetrics and Gynecology. Extensive clinical and laboratory facilities are available to the fellow who will work under the supervision of Dr. A. E. Rakoff, Clinical Professor of Obstetric and Gynecologic Endocrinology, and members of the Endocrine Division of the Department. Stipend $1800 per year. For further details write to Lewis C. Scheffey, M.D., Professor of Obstetrics and Gynecology, Head of Department and Director of Division of Gynecology, Jefferson Medical College and Hospital, Philadelphia 7, Pa.

The 1950 Meeting of the Association for the Study of Internal Secretions

The Thirty-Second Annual Meeting of The Association for the Study of Internal Secretions will be held at the Sir Francis Drake Hotel, Friday and Saturday, June 23 and 24, 1950, in San Francisco, California.

The Committee on Local Arrangements is comprised of Dr. Hans Lisser, Chairman, and Drs. Leslie L. Bennett, Roberto F. Escamilla, Minnie B. Goldberg, Gilbert S. Gordan, and Laurance W. Kinsell.

Hotel accommodations will be difficult to secure on short notice; therefore, members are urged to make their reservations at once. All requests must be addressed to: Dr. William Howard Rustad, American Medical Association (Hotel Committee), Room 200, Civic Auditorium, San Francisco, California.

The scientific sessions will be held in the Empire Room of the Sir Francis Drake, and registration will be on the same floor. The annual dinner will be held in the Empire Room on Friday, June 23, at 7:30 P.M., preceded by cocktails at 6:30 P.M.

Those wishing to present papers, which will be limited to ten minutes, should send title and four copies of an abstract of not more than 200 words, to Edward A. Doisy, M.D., St. Louis University School of Medicine, 1402 South Grand Avenue, St. Louis, Missouri, not later than *March 1, 1950. It is imperative that the abstracts be informative and complete with results and conclusions in order that they may be of reference value and suitable for printing in the program and journals of the Association.* Names of non-members who are co-authors must be followed by the words "by invitation," and the principal degree of each author.

Nominations for the Ayerst, McKenna and Harrison Fellowship, the Schering Fellowship in Endocrinology, the Squibb Award, and the Ciba Award should be made on special application forms which may be obtained from the Secretary-Treasurer, Henry H. Turner, M.D., 1200 North Walker Street, Oklahoma City 3, Oklahoma, and filed with the Secretary not later than *March 15, 1950.*

The 1950 Awards of the Association for the Study of Internal Secretions

FELLOWSHIPS

The Ayerst, McKenna and Harrison Fellowship

The Ayerst, McKenna and Harrison Fellowship was first awarded in 1947 to Dr. Samuel Dvoskin and in 1948 to Dr. Ernest M. Brown, Jr. Dr. Brown was re-elected for the Fellowship in 1949.

The Schering Fellowship in Endocrinology

The Schering Fellowship in Endocrinology was established in 1949 and the first recipient was Dr. D. Lawrence Wilson.

Association Fellowships are designed to assist men or women of exceptional promise in their progress toward a scientific career in endocrinology. Fellowships may be awarded to an individual who possesses the Ph.D or M.D. degree or to a candidate for either of these degrees. The stipend, which will not exceed $2,500, may be divided into two Fellowships in varying amounts in accordance with the qualifications of the appointee. The Committee will, in reviewing the proposed program of study, consider the amount of time which the Fellow intends to spend in course work and/or teaching. He must present evidence of scientific ability as attested by studies completed or in progress and/or the recommendation of responsible individuals. He must submit a program of proposed study, indicate one or more institutions where the proposed program will be followed, and submit statements of approval from the investigators with whom he proposes to conduct his research. He must serve full time if awarded a Fellowship. A small amount of time (10 to 15 per cent) may be allotted for course work or for participation in teaching, the latter purely on a voluntary basis.

AWARDS

The E. R. Squibb and Sons Award

The E. R. Squibb & Sons Award of $1,000 was established in 1939, and was given first in 1940 to Dr. George W. Corner; in 1941 to Dr. Philip E. Smith; in 1942 to Dr. Fred C. Koch; in 1944 to Dr. E. A. Doisy; in 1945

to Dr. E. C. Kendall; in 1946 to Dr. Carl G. Hartman; in 1947 to Drs. Carl F. and Gerty T. Cori; in 1948 to Dr. Fuller Albright; and in 1949 to Dr. Herbert M. Evans. In 1943 no award was given. A special committee of five members of the Association selects the recipient from among investigators in the United States or Canada, on the basis of outstanding contributions to endocrinology.

The Ciba Award

The Ciba Award to recognize the meritorious accomplishment of an investigator not more than 35 years of age in the field of clinical or preclinical endocrinology was established in 1942, but no recipient was selected in 1942 or 1943. In 1944 the award was presented to Dr. E. B. Astwood; in 1945 to Dr. Jane A. Russell; in 1946 to Dr. Martin M. Hoffman; in 1947 to Dr. Choh Hao Li; in 1948 to Dr. Carl Heller; and in 1949 to Dr. George Sayers. The Award is for $1,200. If within twenty-four months of the date of the Award, the recipient should choose to use it toward further study in a laboratory other than that in which he is at present working, it will be increased to $1,800.

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NOMINATIONS

Each member has the privilege of making one nomination for each Fellowship or Award. A nomination should be accompanied by a statement of the importance of the nominee's contributions to, or interest in endocrinology and by a bibliography of the nominee's most important publications, with reprints if possible. The nominations should be made on *special application forms* which may be obtained from the *Secretary*, Dr. Henry H. Turner, 1200 North Walker Street, Oklahoma City, Oklahoma, and returned to him not later than *March 15, 1950*.